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A LABORATORY METHOD FOR TESTING THE TOXICITY OF PROTECTIVE FUNGICIDES

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INTRODUCTION.

FOR many years Bordeaux mixture and lime-sulphur have been the two standard protective fungicides, representing respectively two large groups, the copper- and the sulphur-containing fungicides. Each has, however, certain disadvantages as a spray for apple and pear trees. With Bordeaux mixture there is always a risk of russetting the fruit and scorching the leaves, even at dilutions containing as little as 0.05% copper. Lime-sulphur, on the other hand, frequently causes the dropping of fruit and leaves, especially when used post-blossom at 1% in hot weather. This tendency to cause damage has become increased in recent years by methods of spraying in which the fluids are delivered as driving sprays under high pressure.

There is thus a need either for the development of new fungicides or for the improvement of the old ones. Such improvement might be brought about by preparing the toxic element in some other form. Alternative preparations of copper and sulphur are already available as so-called "colloidal copper" and "colloidal sulphur", and organic compounds represent a wide field for further development provided they can ultimately be economically used.

Past experience has shown that field testing of protective fungicides for fruit trees can be long and costly. The most important fungus disease of apples is Scab (*Venturia inaequalis* (Cooke) Wint.). It develops slowly, infection becoming severe only when climatic conditions favour it, and a whole year must elapse to complete a field test of any one preparation. In dry seasons, there may be insufficient infection to provide a reliable test, so that much of the work yields inconclusive results and has therefore to be repeated. The work described in the present paper was undertaken to ascertain whether a laboratory method could be devised that would enable time and money to be saved and at least permit reliable comparisons to be made as to the probable relative toxicities of old and of new protective fungicides.

It is not claimed that, by the use of the method proposed here, field trials are rendered unnecessary, but that much can be saved by eliminating unsatisfactory preparations at an early stage. Moreover, some idea can be obtained as to the minimum dilution toxic to the spores of the fungus. It is obvious that a method in which glass slides are used is limited in scope and may not be fully

satisfactory because reactions between fungicide and leaf, or between fungus and leaf, are not involved. Such reactions, however, will be encountered when promising materials are carried beyond preliminary laboratory tests to field trials.

LITERATURE.

For testing protectants many workers have used methods in which the fungus was brought into contact with the fungicide in suspension or in solution (Burrill (1), Hamilton (5), Liming (6), Uppal (15)), or in an agar culture-medium (Shapovalov (*), Palmiter (13)). Although these methods give a certain amount of useful information, they do not give any indication of what may happen when the fungicides are sprayed on to a plant and allowed to dry. Doran (3) has shown that when conidia of *Venturia inaequalis* on the surface of an apple are sprayed with lime-sulphur solution, they are killed at a much lower concentration than when put on the dry deposit. Moreover, with many protective fungicides, great changes take place on drying and exposure to air. The only methods suitable for comparing protectants, therefore, are those that make use of the dry deposit.

Burrill (1), in 1907, carried out germination tests on two sprayed cover-glasses, but the first to evolve a method on this basis were Reddick and Wallace (14) in 1910. It consisted of spraying slides or cover-glasses with a material of known formula, drying and exposing them to the air, and putting spores in a drop of meteoric water on the dry deposit. The method used in 1911 by Wallace, Blodgett and Hesler (16) was essentially the same, but special precautions were taken to ensure that the spraying was as uniform as possible. Attention was paid also to standardizing the spore-concentration, and controls were retained on the unsprayed half of each slide. The spores used were from Scab-lesions on apple leaves or fruit. Doran (3), in 1922, used a similar method, incubating at 14° C., a temperature that he found most favourable for the germination of conidia of *V. inaequalis*. This was followed in 1928 by the combined work of Butler and Doran (2) who used this method and obtained results on the toxicity to *V. inaequalis* of a number of arsenical preparations used alone and in combination with lime-sulphur. These authors refer to much useful literature on the subject. This method was further developed by McCallan (9) in 1930. He took special precautions to distribute the spray evenly over the surface of the slides by using a hand-atomizer that delivered a fine, uniform, mist-like spray, and he claimed that, provided a certain minimum amount was applied, increase of it did not appreciably increase fungicidal efficiency. The spore-suspension was applied as a drop from a pipette that delivered 20 drops per c.c. Only one fungicide was placed in each moist

* The work of M. Shapovalov is unpublished, but is described by Morse (12, p. 178).

chamber. Martin (8), in 1932, surveyed the problems connected with both direct and protective fungicides, and, concerning the latter, he stressed the importance of temperature, oxygen relationships and the biological state of the spores. He issued the warning that "laboratory trials are dangerous tools and their value depends on what attention is paid to the tacit assumptions which they involve".

A rather different method, with a limited application, was evolved by Goodwin, Salmon and Ware (4) in 1929, in which a water-suspension of zoospores of the Hop Downy Mildew (*Pseudoperonospora Humuli*) was placed on a dry spray-deposit and observed under the microscope. If the zoospores burst in a short time a good fungicide was indicated. This method seems suitable for use only with fungi that produce zoospores, and is therefore useless for the large majority of parasitic fungi. It is unsafe to conclude that a fungicide that is effective against one fungus will also be effective against others, hence each fungicide should be tested against the specific fungus it is required to combat.

GENERAL PRINCIPLES INVOLVED.

The method described below was developed for testing fungicides to control Apple Scab, and involved the use of conidia of the *Fusicladium* stage of the fungus *Venturia inaequalis* (Cooke) Wint. It was felt that the methods of other workers were not quite suitable for this purpose, mainly because the amount of spray applied was not sufficiently controlled. Any method that depends on visual estimation of uniform application must be open to criticism, especially when spray-substances having different wetting and spreading properties are used. Accordingly, a method of application was sought that would ensure precision in the amount of spray-material used in each experiment. This was obtained by applying a standard volume of diluted fungicide to a standard area of a standard surface. Glass was chosen as the most suitable surface because it is transparent and also enabled the method to be used throughout the year. With the possibility in mind of a reaction in the field between fungicide and leaf, which might influence fungicidal value, Marsh (7) used living leaves as well as cellulose-coated glass slides, and applied the spray-fluids with a precision spraying apparatus. He found that the living leaf "proved less flattering to the fungicide" than did the slide.

From the start (10), the guiding principles underlying the development of the method described below were: (a) standardization as far as possible of every phase in the process, to facilitate accurate repetition when desirable and to give uniform conditions for every preparation tested; (b) simulation, as far as possible within the terms of (a), of the conditions encountered by fungus and fungicide in the field.

THE METHOD.

With a fine-bore glass pipette, 0.015 c.c. of the fungicide at the required dilution is applied to each of a number of circular areas 15 mm. in diameter, marked on a 3" x 1" glass slide. The fungicide is carefully spread to the edge of each area with a glass needle so that the area is completely covered by a film of liquid. This film is allowed to dry at room temperature, and is stored overnight at 20° C. A suspension in sterile distilled water of conidia from a pure culture is adjusted to a suitable concentration and is then dropped from a pipette at the rate of 0.04 c.c. on each area bearing the dry deposit of fungicide, and also on a number of clean areas as controls. The suspension is spread with a glass needle to cover each area completely, and the slides are placed in moist chambers, at 20° C., for at least twenty-four hours. The amount of germination is then recorded. When desired, washing with water to simulate the action of rain, is carried out after the fungicide has dried and before the suspension of conidia is applied.

Certain features of this method have required special study and are worthy of more detailed description. This will be found in the following paragraphs.

GLASSWARE.

Thin glass microscope slides of good quality were used. A circle of 15 mm. diameter was described with a writing diamond in the middle of each slide by rotating it in a lathe. Later, to save space and time, three such circles were drawn on each slide. Such slides can be obtained ready prepared, if desired, from commercial sources. It was found that 0.015 c.c. of liquid will cover each circular area and form an unbroken film on drying, except with liquids or suspensions of very high surface tension. With these, a small amount of some suitable "wetter" can be added to lower the surface tension, but in these experiments a different form of slide was used, the circular areas having a matt surface, produced by sand-blasting, thus reducing the surface tension between the liquid and the glass. The rough surface, however, is a disadvantage in that it renders it difficult to see whether or not the conidia have germinated. Staining with Gentian Violet or Cotton Blue makes matters easier.

The special pipettes used were made from glass tubes of fine bore, each fitted with a small rubber teat (such as are found in the B.D.H. Capillator Case for determining pH); they were graduated to deliver the required amounts.

The moist chambers were large, shallow Petri dishes, 6 in. in diameter, in which was placed sufficient distilled water to cover the bottom of the dish. Racks to support the slides were made by bending glass rod to the required shape.

All the glassware used was very carefully cleaned, especially the slides, to ensure that no foreign matter was present. Minute traces of grease are sufficient to prevent the fungicides from spreading over the prescribed area. Cleaning

involved the use of the following chemicals in order—warm dilute nitric acid (to remove fungicide-deposit); hot, 2.5% caustic soda (to remove grease); chromic acid cleaning solution (to remove organic matter); and ether (to remove any fats remaining). The slides were washed with water between each two operations, and were dried before being put into ether. Finally, they were transferred from ether to absolute alcohol, where they remained until required. Each was flamed and allowed to cool before the spray-fluid was applied. After tests in which organic fungicides had been used, the slides were washed in acetone, as this removes most of these materials.

APPLICATION OF FUNGICIDE.

With many fungicides, satisfactory cover of the test area can be obtained with dilutions in distilled water, but it was found that the suspension obtained in water with certain insoluble organic compounds tended to "cream", making uniform distribution of the fungicide almost impossible. This was overcome by dissolving the pure compound in up to 2% acetone (usually 1%) and then emulsifying in a hand-operated machine with a 0.1% solution of "goulac" (sulphite lye). Goulac had been tested and found, up to at least 4%, to have no fungicidal effect on the conidia.

WASHING THE SPRAY-DEPOSIT.

The earlier results, obtained with lime-sulphur and Bordeaux mixture, showed that, while the toxicity of various concentrations of the former approximated to that found in the field, that of Bordeaux mixture did not. Thus, a decrease in the concentration of lime-sulphur from 2.0% to 0.5% resulted in a great increase in the percentage of germinated conidia, while Bordeaux mixture did not allow free germination even when used as dilute as to contain only 0.025% copper sulphate (hydrated lime being present at the rate of three parts to two parts copper sulphate), which is a much lower concentration than that normally employed in the field. It seemed probable, therefore, that only a minute trace of a copper salt was required for toxicity, yet under field conditions, any considerable reduction in the copper sulphate content of Bordeaux mixture has resulted in decrease in the efficiency of this spray against Apple Scab. Under field conditions, a number of agents tend to reduce the amount of the deposit, the chief being rain. The rate at which rain water removes the deposit will influence the period during which the spray will remain effective. An attempt was made, therefore, by modifying the laboratory method, to obtain some indication of the duration of this period. For this purpose, a standard method of washing the deposit, after twenty-four hours' drying, was introduced.

An early method was to suspend the slides vertically from glass rods so that they were completely immersed in a large glass bath of distilled water. This method was preferred to that of washing with running distilled water chiefly for economy's sake, although the latter method would probably simulate more closely the action of rain. In the first experiment the distilled water was saturated with CO_2 , but this proved so drastic that it entirely removed the deposit in a relatively short time. In subsequent experiments, therefore, fresh distilled water was used. Slides were removed at various intervals and allowed to dry. When a whole series was completed, all the slides were stored overnight in an incubator (20°C.), and next morning a spore-suspension was applied.

Later, it was found more satisfactory to submerge the slides in the water on horizontal glass racks with the surfaces bearing the spray-deposit facing downwards. The type of glass rack ultimately used could be gently rocked, and this prevented localized concentration in the water of any substance that might come from the deposit. This rocking also simulates to some degree the action of water flowing over the surface of a sprayed leaf. A rocking apparatus involving a pendulum, actuated by a water-jet, was set up and adjusted to give a standard number of swings of known amplitude per minute. This standard was found to vary somewhat with fluctuations in water-pressure and with the loading of the racks, hence an electric induction motor, driving a cam, was substituted as the power-unit as it could be run at constant speed irrespective of loading. The apparatus is illustrated in Fig. 1.

By this washing method, it was found that a Bordeaux mixture containing 0.025% copper sulphate remained toxic after two hours' washing, while one containing only 0.0125% copper sulphate was not effective after one hour's washing.

Lime-sulphur at 1% remained toxic after two hours' washing, but at 0.2% it was not effective, even unwashed. Reference to the results in Table IV to be found on page 263 shows that lime-sulphur at 0.33% (i.e. approximately 0.067% by weight polysulphide sulphur) was the lowest concentration effective, unwashed.

The figures obtained from these experiments are set out in Tables I and II.

The experiment with 0.0125% Bordeaux mixture was repeated on two other occasions and gave a similar result.

Very similar results had been obtained in the earlier experiments while the method was being developed.

All potential protective fungicides should be subjected to this washing test after the toxic limit of the unwashed deposit has been determined. If the concentration representing this toxic limit has to be greatly increased for the deposit to be effective after two hours' washing, the fungicide probably will not long remain effective under field conditions.

TABLE I.

Results of washing Bordeaux mixture deposits.

Fungicide and Washing Period.	Germination of Conidia.			Category.*
	Germ.	Inhib.	Ungerm.	
No fungicide (control)	100	0	9	5
0.025% Bordeaux mixture (0.006% Cu). Washed 2 hours ..	No germination.			1
Washed 1 hour ..	..	..		1
Washed $\frac{1}{2}$ hour ..	..	..		1
Unwashed ..	..	..		1
0.0125% Bordeaux mixture (0.003% Cu). Washed 2 hours ..	41	32	70	2-3
Washed 1 hour ..	8	18	95	1-2
Washed $\frac{1}{2}$ hour ..	No germination.			1
Unwashed ..	..	..		1

* See under "Methods of Assessing Results" on page 261.

TABLE II.

Results of washing lime-sulphur deposits.

Fungicide and Washing Period.				Germination of Conidia.	Category.*
No fungicide (control)				Fully germinated.	5
0.04% lime-sulphur. Washed 2 hours ..	..	..			5
Washed 1 hour ..	..	..			5
Unwashed ..	..	..			5
0.2% lime-sulphur. Washed 2 hours ..	..	..			5
Washed 1 hour ..	..	..			5
Unwashed ..	..	..		Mainly germinated with short germ-tubes.	4
1.0% lime-sulphur. Washed 2 hours ..	..	..		No germination.	1
Washed 1 hour ..	..	..			1
Unwashed ..	..	..			1

* See under "Methods of Assessing Results" on page 261.

STANDARDIZATION OF FUNGUS.

As it is not easy to obtain natural supplies of conidia of *V. inaequalis* all the year round, and as naturally occurring conidia tend to be very variable in germination, they must be obtained from artificial cultures. In the present work the cultures were started from single conidia obtained from Scab-spots on the leaves of Bramley's Seedling, and a fresh isolation was made each season. This fungus does not readily produce conidia on many media, and its growth-rate is slow even on a favourable medium. An agar plate medium proved unsuitable owing to the difficulty of removing the conidia from the surface of the medium with sterile water; hence it was discarded. Agar films were produced by horizontally rotating test-tubes each containing a small quantity of warm agar. Although these yielded a satisfactory suspension of conidia in sterile water, their use was ultimately given up because they were difficult to handle, and pieces of the film became detached. Pieces of sterilized apple wood in test-tubes were found to be the most suitable medium, as conidia were produced plentifully and were easily detached by water. These pieces were obtained by splitting three-inch lengths of three- or four-year-old apple branches from which the bark had been peeled, and they were then placed singly in test-tubes with a little water and autoclaved.

At first the pieces of wood were inoculated by spraying from an atomizer with a suspension of conidia in distilled water, but later it was found more satisfactory simply to pour into the tube a quantity of the suspension, which was then shaken up so that the surface of the wood was uniformly wetted. Trials were made with suspensions of conidia in nutrient solutions instead of in distilled water. The solutions tested contained 10% dextrose, 10% laevulose, 10% sucrose, 5% sucrose, 1% malt extract and 0.5% asparagin. Although slight differences were recorded in the numbers of conidia produced and in their capacity for germination, they were too small to be significant.

The pieces of wood must be kept almost saturated with water, for if they become too dry the fungus grows very slowly, and if too wet, the fungus produces a mass of mycelium and few conidia. The optimum moisture conditions can be found only by experience, but if the wood is saturated with water before autoclaving and about 1 c.c. of conidia-suspension is introduced when inoculating, the resulting culture should give sufficient conidia in fourteen days. If the cultures are shaken daily, conidia are produced more plentifully than if the tubes are not disturbed.

Before experience of this method had gone very far, considerable variations in germination capacity and germ-tube vigour were found among different conidia-suspensions. At first this was thought to be connected with the age of the conidia and cultures of various ages were tested. It was then found that while cultures fourteen days old produced large numbers of conidia that

germinated well, very old cultures gave a high proportion of spores that did not germinate. All the cultures subsequently used, therefore, were about fourteen days old. The percentage of conidia that germinated, however, still varied very much between one culture and another, and eventually it was found that nutriment was extracted from the pieces of wood while the suspension was being made, and that the amount extracted varied with each occasion. In order to counteract this, the conidia were washed. The suspensions were centrifuged until the conidia collected at the bottom of the tube, after which the water was poured off and the tube was refilled with distilled water. This process was repeated three times, and the suspension was finally adjusted to contain approximately 50 conidia per field of 2 mm. diameter. A suspension prepared in this way gave excellent germination and very consistent results. Recently, however, a series of these suspensions showed no germination, and the cause was finally traced to the distilled water, which had been obtained from a silver still. A minute trace of some toxic substance must have been introduced from this still, for when the water was re-distilled from a glass vessel, ready germination was restored. Conidia, when suspended in distilled water with a trace of nutriment, appear to be able to withstand the toxic effects of the fungicide-deposit better than when they are in pure distilled water.

APPLICATION OF CONIDIA-SUSPENSION.

In the method as described in a previous paper (10), 0.05 c.c. of the conidia-suspension was put on each area. Later, it was found that this amount sometimes overflowed the delimited area and this undesirable effect was successfully overcome by reducing the volume to 0.04 c.c.

METHODS OF ASSESSING RESULTS.

It was necessary to examine the conidia with a $\frac{1}{8}$ " objective and $\times 6$ ocular because the density of the deposit at the higher concentrations made it very difficult to see them with a low power. Ten fields or traversed portions, each containing approximately ten conidia, were taken at random on each circular area and the germination was recorded. Three categories of conidia were counted, (a) those ungerminated, (b) those with a germ-tube not longer than the conidium (inhibited* after initial germination), (c) those with a germ-tube longer than the conidium. The first examination was generally made after twenty-four hours' incubation, but some of the replicates were always left in the incubator for at least forty-eight hours to ensure as complete germination as possible.

When a large number of substances have to be dealt with, it is desirable to have a more rapid method than that of counting individual conidia, especially

* These conidia were not "late starters" for they were subsequently examined at intervals and no further growth was observed.

for preliminary sorting, when only wide differences are needed. Hence germination was classified by inspection into categories as follows:—

1. All ungerminated or with very few inhibited.
2. A mixture of ungerminated and inhibited in roughly equal proportions.
3. Inhibited predominating, with some ungerminated and some germinated.
4. Germinated predominating, with a considerable number inhibited and a few ungerminated.
5. Practically all germinated.

Considerable experience of the counting method is needed before accuracy in estimating germination by this category method is achieved. The counting method is essential also for the critical determination of the minimum toxic concentration of any fungicide.

RESULTS.

That the results of each experiment in comparable series are in close agreement is demonstrated by the category figures obtained from specific tests repeated several times. These will be found in Table III.

TABLE III.

Experiment No.		1.	2.	3.	4.	5.
Series I. No fungicide (control)	Colloidal copper preparation (6% Cu)	5	5	5	5	5
	0.0003% Cu			4.5		
	0.0004% Cu			3		
	0.0006% Cu	2-3	1-2	1-2	1	3
	0.0018% Cu		1			
	0.003% Cu		1		1	
	0.006% Cu	1			1	1
	0.06% Cu	1				
Series II. No fungicide (control)	Copper sulphate	5	4	5	5	
	0.0003% Cu		1	1-2	1-2	
	0.0016% Cu	1				
	0.0025% Cu		1	1	1	
	0.0031% Cu	1				
	0.006% Cu	1				
	0.025% Cu		1	1	1	
	0.00022% Zn		3	5	5	
	0.0022% Zn		2	3	4	
	0.022% Zn		1	1	1	
Zinc sulphate						

Table IV contains a selection from the results so far obtained by the use of the method described, the deposit of fungicide in every case being unwashed.

The headings of the three columns in the Table are to be interpreted as follows:—

Spray-fluid not effective: Germination in categories 4 and 5, germinated conidia predominant.

Spray-fluid partially effective: Germination in categories 2 and 3, inhibited and ungerminated spores predominant, and only a few germinated conidia.

Spray-fluid effective: Germination up to but not including category 2, only a small proportion of inhibited and no germinated conidia being included.

TABLE IV.

Preparation.	Not effective. (Cat. 4-5) at :	Partially effective. (Cat. 2-3) at :	Effective (Cat. 1) at :
<i>Containing copper :</i>			
Copper sulphate		0.0003% Cu	0.0016% Cu
Bordeaux mixture			0.003% Cu
Colloidal copper preparation (6% Cu) ..	0.00045% Cu	0.0006% Cu	0.0018% Cu
<i>Containing zinc :</i>			
Zinc sulphate	0.002% Zn		0.022% Zn
Zinc-lime	0.41% Zn		0.81% Zn
Zinc-lime-"goulac" preparation (6.7% Zn)		0.11% Zn	0.23% Zn
<i>Containing sulphur :</i>			
Lime-sulphur	0.04% S†		0.067% S†
A 40% sulphur-dispersion A	0.001% S	{ 0.01% S and 0.1% S 0.1% S 0.1% S	
" " " B	0.01% S		
" " " C	0.001% S		
<i>Organic compounds :</i>			
Para-acetoxypheylisothiocyanate ..	0.001%	{ 0.01% and 0.1% 0.1%	
α -Chloro- β -naphthol	0.01%		
8-Hydroxyquinoline			0.001%*
2-Mercaptobenzthiozole	0.001%	0.005%	0.01%
Methyl " dixanthogen "	0.1%		
β -naphthol	0.1%		
Salicylanilide	0.002%	0.003%	0.004%
Tetramethylthiuram sulphide	Inconclusive		0.005%
Tetramethylthiuram disulphide	0.0001%		0.0005%
Thiodiphenylamine	0.05%		0.1%

* Lowest concentration tested; the other figures in this column are the lowest effective concentrations.

† S in this case is sulphur in polysulphide form.

DISCUSSION OF RESULTS.

The results obtained by this method suggest that it is reliable for comparing fungicides in the laboratory. Whether there is any correlation between them and those that would be obtained in the field is a subject for future investigation.

The following comparisons show that it is unlikely that a single numerical factor will be found by which the effective concentration of any fungicide determined in the laboratory can be multiplied to give the concentration necessary for effective use in the field. With lime-sulphur, the lowest effective concentration in the field (1%) is three times that of the lowest effective concentration in the laboratory (0.33%, unwashed). Similar comparison of Bordeaux mixture in the field (0.2% copper sulphate) and in the laboratory (0.0125% copper sulphate) gives a factor of sixteen. Even when the concentration (0.025% copper sulphate) effective after two hours' washing is used for the comparison, the factor is still as high as eight. It seems very probable, therefore, that with widely differing fungicides such as lime-sulphur and Bordeaux mixture, a different ratio between the results obtained in laboratory and field will be found for each.

With organic compounds, concentrations above 0.1% were not considered, because the high cost of these compounds renders their use prohibitive unless they are effective at low concentrations. The promising organic compounds among those tested, therefore, were 8-hydroxyquinoline, salicylanilide, tetramethylthiuram sulphide and tetramethylthiuram disulphide. In a field trial in 1935 (11), a spray containing 1% of a 15% dispersion of tetramethylthiuram sulphide, though more effective than one containing 1% of a 25% dispersion of salicylanilide, was not as effective as the standard lime-sulphur spray (2.5% pre-blossom; 1% post-blossom) in controlling Apple Scab. Since, in laboratory trials, tetramethylthiuram disulphide proved more fungicidal than tetramethylthiuram sulphide, it seemed well worth a field trial. This compound has therefore been included in one in 1937, and 8-hydroxyquinoline will be tested as opportunity offers.

SUMMARY.

1. The literature relating to laboratory methods for testing protective fungicides is reviewed.

2. The following method has been devised and tested: With a fine-bore pipette, quantities of 0.015 c.c. each of the liquid fungicide are spread uniformly over circular areas, each 15 mm. in diameter, delimited on glass slides. When the liquid has evaporated and the deposit is dry, 0.04 c.c. of a suspension in water of washed conidia from a pure culture of *Venturia inaequalis* is applied in a similar manner, and the slides are then stored in a saturated atmosphere

at 20° C. At intervals counts of germination are made. When desired, the spray-deposit is washed with water before the conidia-suspension is applied.

3. A method of growing *Venturia inaequalis* in pure culture for the production of conidia is detailed, as well as one for freeing the conidia-suspension from nutriment by centrifuging.

4. A method is presented for rapidly assessing the results of treatments by classifying the mass appearance of conidia-germination into five categories.

5. The results of a number of tests with copper- and sulphur-containing inorganic substances, and also with certain organic compounds are tabulated and discussed. Amongst the last-named, tetramethylthiuram disulphide was found to be very highly fungicidal.

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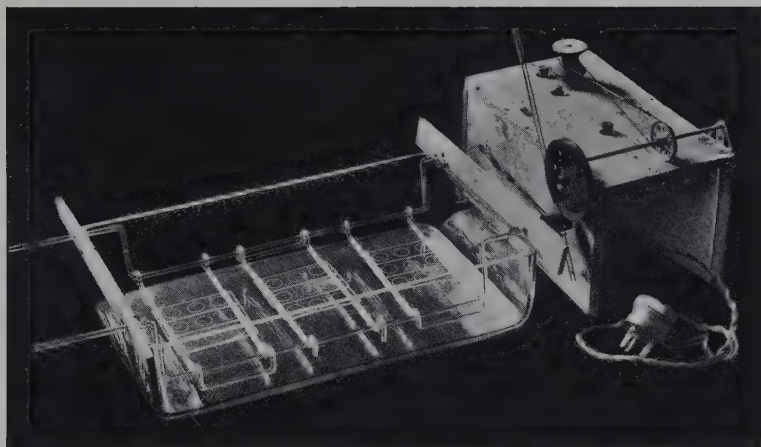
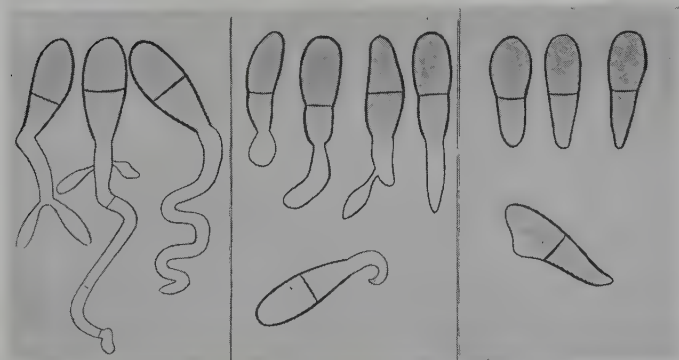


FIG. 1.

Apparatus for washing deposit of spray-fluid on glass slides. An electric induction motor is housed in the box and rotates the reel from which the drive is transmitted to the cam by a belt. The glass rod is held in the grooved edge of the cam-wheel and transmits the rocking motion to the racks.

(approx. $\frac{1}{3}$ actual size.)



[By courtesy of the Cambridge University Press.]

FIG. 2.

Illustrating conidia of *Venturia inaequalis* classed as: germinated (left), inhibited (centre) and ungerminated (right).

STUDIES IN INCOMPATIBILITY BETWEEN STOCK AND SCION, WITH SPECIAL REFERENCE TO CERTAIN DECIDUOUS FRUIT TREES*

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I. INTRODUCTION.

The problem of incompatibility between stock and scion is one which has puzzled propagators and pomologists all over the world ever since grafting and budding began to be employed. Thus, in China, as early as about A.D. 500, Chia Su-lih (7) wrote in his book *Chi Ming Yao Suh* (Methods of Progressive Farming) that the pear, *Pyrus serotina* Rehd., is best suited on "Tu Li", *Pyrus phaeocarpa* Rehd., and that the bark of the scion must be brought into close contact with that of the stock in order to get good union. He also mentioned that the plum, *Prunus salicina* Lindl., can successfully be grafted on peach stocks, *Prunus persica* Lindl., and will bear prolifically, whereas the peach usually fails to grow on plum stocks, *Prunus salicina* Lindl. Accounts in old French and English literature also mention the phenomenon of incompatibility. Thus, Thomas Andrew Knight (32), writing in 1812 on the effect of different kinds of stocks in grafting, stated that plum stocks are best adapted to the peach, and apricot stocks to the Moorpark Apricot. He said that his Moorpark Apricot budded on apricot stock had smooth bark and a polished surface, and that the whole tree presented a greater degree of health and vigour than did trees budded on any other stock.

This problem of incompatibility is a serious handicap to the nurseryman in choosing suitable stocks. From the point of view of the practical grower, it is a disadvantage when big trees, on reaching bearing age, are suddenly broken off at the union in a storm. It is also fairly common to see 8- or 10-year-old trees gradually decline both in fruiting and in vegetative vigour so that finally they have to be grubbed.

Most fruit trees are budded or grafted, i.e. are made up by combining a scion with a stock. If there were any abnormal manifestations or obstructions at the union causing trouble to the vital growth-processes of the tree, then either the scion or the stock effect may be interfered with. Therefore, a closer

* The present paper is an abridgment of a Thesis (presented in August 1937) approved for the Degree of Doctor of Philosophy in the University of London.

study of the symptoms and possible causes of incompatibility may afford some explanation of certain stock-scion relations.

Survey of the literature. Since the end of the nineteenth century many attempts have been made to explain the problems of incompatibility. Thus, Waugh (64), Proebsting (43, 44), Heppner and McCallum (23, 24) and Bradford and Sitton (4), attributed incompatibility chiefly to structural weakness and abnormal deposits at the union. Daniel (10), Gleisberg (12), Crafts (9), and Mendel (39), attributed it mainly to differences in the anatomy of stock and scion, while Guignard (15), Rives (48), Green (14), Hofmann (25), Proebsting and Barger (45), Haas and Halma (16) and Toxopeus (61), believe that the cause is of biochemical origin. Hafekost (17), working with pears grafted on East Malling quince selections D, E, F, G, stated that there was a difference in the power of protoplasmic reaction between stock and scion, resulting in a higher or lower concentration of the cell sap. Kostoff (33, 34, 35), on the other hand, from his results with intergeneric grafts of Solanaceae, concluded that the toxins produced by different antibodies induced by the presence of antigens in different kinds of plants are responsible for incompatibility. None of these interpretations has been finally accepted, and up to the present there is still no method of predetermining the compatibility or otherwise of any given combination of stock and scion.

Botanical relationship not a reliable guide. Older conceptions regarded botanical relationship as a fair guide to the compatibility of the stock and scion. Baltet (3) held that plants belonging to the same botanical group may successfully be grafted. But plants of different species or even of different genera, such as lilac (*Syringa*) on a kind of privet (*Ligustrum lucidum* Lindl.), or Citrus on *Poncirus trifoliata* Lindl., are commonly and successfully worked in China. On the other hand, Hatton (19) found that different selections of quince stocks belonging to the same species (*Cydonia vulgaris* Lindl.), showed marked differences in regard to their suitability for pears. Later reports from East Malling (20, 21, 22) concerning pears, plums, and peaches, also showed clearly that someplum varieties (*Prunus domestica* L.) show a less degree of compatibility with Common Plum stock (*Prunus domestica* L.) than with a Cherry-Plum selection, Myrobolan B. (*Prunus cerasifera* L.). Very different results have been obtained even among selections bearing the same varietal name, such as St. Julien Plum. Thus, while the peach Hale's Early has grown satisfactorily on St. Julien A and St. Julien C, it has proved completely incompatible with St. Julien K. From this evidence it would appear that incompatibility cannot be directly correlated with any taxonomical relationships.

Disease symptoms and signs of incompatibility must be distinguished. Quite often a tree dies because of infection with some kind of disease-producing organism

at the union or in the rootstock portion of the tree. Again, it is not uncommon for a tree to die suddenly from excessive heat or from waterlogging. Riker and others (47) have clearly shown how overgrowths and other malformations resembling Crown Galls at the union in root-grafted apple trees may result as an after-effect of wounding. Similarly, Rawlins and Horne (46) have described how in cherries the wound made in grafting may act as a place of entry for disease, and Wormald (65) has proved that a wound is the primary place of entry for the Bacterial Die-Back organism in plum trees. Pitmaston Duchess pear has been looked upon as a compatible scion on Quince C, but in the summer of 1936, as many as 50 per cent. of such grafted trees died within a short period in the nursery at East Malling. On examining the dead trees, the bark of the scion was found to be badly blistered, and many cells inside the wood were blackened. The stock portion was healthy and white inside. It was found that two trees were badly cankered above the union.

Percentage of take not a reliable indication of incompatibility. Percentage of bud and graft take is sometimes very variable. Climatic and environmental conditions during the time of budding and grafting, the condition and size of the stock, the maturity of the scion wood, the date and method of propagation are all factors which influence the percentage of take. Work conducted at East Malling by Amos and others (1) has shown that the percentage of bud take cannot always be depended upon as a fair indication of incompatibility. For instance, Czar Plum budded on Common Plum showed about 37% take in 1933, 70% in 1934, and 0% in 1935. Similarly, Pitmaston Duchess pear budded on Quince F had 100% take in 1934 but only 62% in 1935.

Again, the percentage of bud take may vary considerably according to the date of budding and the method of handling the buds. To illustrate this point, buds of Hale's Early Peach were inserted into three different plum stocks at weekly intervals from June 24th to August 26th, 1936. Those on one of the stocks, Common Plum, showed striking variations in the percentage take according to the date of budding. Thus, the numbers of buds still alive on March 15th, 1937, of each ten, budded on three stocks on the dates mentioned, was as follows :

	24/6	1/7	8/7	15/7	22/7	29/7	5/8	12/8	19/8	26/8
Common Plum	.. 0	0	1	3	4	1	3	3	6	8
Brompton	.. 8	10	10	10	9	10	10	10	9	9
Myrobolan B	.. 7	8	8	10	10	8	10	10	10	10

Eventually, all the trees on Common Plum died at the end of May, and, at the end of August 1937, those on Myrobolan B appeared likely to die.

Work in Chekiang, China, (6) has shown that the percentage of bud take of Bartlett and Kieffer pears on *Pyrus calleryana* stocks varies markedly with the

humidity, rainfall and diurnal temperature during the period of budding. It is also known that climatic and environmental conditions influence the manifestations of incompatibility to a great extent, even after the buds have taken and the scions have started growth. Thus, while it is well known that the apple will not normally grow for more than a few seasons when grafted on quince stocks, a recent communication from H. A. Urbina, in Uruguay, states that two varieties, Winter Banana and Reineta de Verano, have been growing well on certain quince stocks for over twenty years, and are still producing about 120-150 kilograms of fruit per tree per annum. Mitchurin (40) states that a lemon tree has been successfully grafted on a pear stock and is still living and growing after six years.

There is some evidence that certain combinations will grow in one locality but may totally fail in another. In such cases it would appear that incompatibility exists, but may not always reveal itself morphologically owing to favourable growth conditions. Unless the condition at the union is closely examined, too hasty a judgment must not be made by looking at the performance of the top growth. It may therefore safely be assumed that the symptoms of incompatibility appear most quickly and in the severest manner when the tree is grown under the most adverse environmental conditions. On the other hand, under favourable conditions, the symptoms may take some time to appear, although incompatibility actually exists in the tree. Argles (2) suggested that "incompatibility may be regarded as an inherent antagonism or discordant association between certain stocks and scions, the cause or causes of failure or abnormalities arising out of the nature of two plants, and the form taken by the failure being governed in greater or less degree by the subsidiary factors of environment and treatment. Thus, under external conditions which favour union and growth, failure will tend to be delayed, while under unfavourable conditions of environment and treatment it will tend to be accelerated."

II. SYMPTOMS OF INCOMPATIBILITY.

In order to study the various manifestations of incompatibility in growth and other internal abnormalities both in supposedly compatible and incompatible grafts and buds, some eighty-six graft combinations of pear, apple, quince, plum, peach, and cherry scion and stock varieties were made in the spring of 1936, and thirty-two bud combinations of pear, quince, plum, and peach scion and stock varieties were made in the summer of that year. The rootstocks were of clonal origin, propagated vegetatively at East Malling. The stock and scion varieties used and mentioned below were selected to represent two main groups, those generally considered compatible and those generally considered incompatible, as described by Amos and others (1) :

Pears :

Compatible combinations :

- Conference on Quince A and C.
- Durondeau on Quince A and C.
- Pitmaston on Quince A and C.

Partially incompatible combinations :

- Conference on Quince F and G.

Incompatible combinations :

- Durondeau on Quince F and G.
- Pitmaston on Quince F and G.

Plums :

Compatible combinations :

- Victoria on Myrobolan B.
- Victoria on Common Plum.
- Czar on Myrobolan B.
- President on Myrobolan B.

Incompatible combinations :

- Czar on Common Plum.
- President on Common Plum.

Peaches :

Compatible combinations :

- Hale's Early on Brompton.
- Hale's Early on St. Julien A and C.

Incompatible combinations :

- Hale's Early on Myrobolan B.
- Hale's Early on St. Julien K.

Cherries :

Compatible combinations :

- Prunus serrulata* on Mazzard.
- Prunus pseudocerasus* on Mazzard.

Incompatible combinations :

- Prunus serrulata* on Mahaleb.
- Prunus pseudocerasus* on Mahaleb.

These combinations were made up in three different ways, viz. scion on stock, stock on scion, and stock on stock, in order to observe any symptoms of incompatibility in the different combinations and particularly to see whether the same symptoms appeared in the reciprocal combinations.

1. *Initial take and subsequent growth of grafts and buds.*

All the above mentioned stocks were of the same age in each comparable group, and were selected for uniformity in the field. The scions and buds were taken from the middle portions of shoots of uniform maturity from trees of the same age.

When grafting, the stocks were arranged in rows so that two replications could be made for every combination. The stocks of the budded trees had been planted in 1935 in randomized blocks, and four replications were made of each combination.

To ensure uniform manipulation all buds and grafts were inserted by the writer at the same height (approximately 6 cm. above ground level) and position (on the north side) on each stock by the same methods, viz. shield, and whip and tongue respectively.* All grafts or buds in each group were inserted on the same day and wax was applied to the grafts. All the after-treatment in the nursery was uniform throughout each group. The weather conditions during grafting and budding in 1936 were normal. The soil moisture in the nursery, as indicated by meter readings, showed that there was no lack of moisture in the soil throughout the growing season following grafting and budding.

(a) *Grafted trees.* At the end of the growing season (December 1936) the new wood growths of each combination were measured. The percentage take and the average growth of new wood for each combination are shown in Tables I-IV:

TABLE I.
Percentage take and Maiden Growths of Pear and Quince Grafts.

Combinations.	No. grafted.	No. take.	% take.	New Wood Growth. cm.	S.E.
Conference on Quince A ..	20	19	95	134.8	13.8
Conference on Quince G ..	20	19	95	109.3	8.9
Durondeau on Quince A ..	20	19	95	100.4	20.1
Durondeau on Quince G ..	20	19	95	160.0	17.6
Quince A on Durondeau† ..	20	18	90	171.9	84.4
Quince G on Durondeau† ..	20	2	10	90.7	36.4
Quince A on Quince A ..	20	16	80	336.3	92.6
Quince A on Quince G ..	20	17	85	625.3	88.8
Quince G on Quince A ..	20	7	35	487.3	120.8
Quince G on Quince G ..	20	19	95	705.2	106.7

* In budding, any small portion of wood that might accompany the bud was not removed before insertion.

† These trees were already budded on Quince A.

TABLE II.

Percentage take and Maiden Growths of Plum and Plum Stock Grafts.

Combinations.	No. grafted.	No. take.	% take.	New Wood Growth. cm.	S.E.
Victoria on Myrobolan B ..	15	15	100	385.0	53.1
Victoria on Common Plum ..	15	14	93	117.5	10.6
Czar on Myrobolan B ..	15	14	93	556.1	87.0
Czar on Common Plum ..	15	7	47	61.7	12.8
Myrobolan B on Czar* ..	20	17	85	460.6	53.3
Common Plum on Czar* ..	20	8	40	81.8	13.1
Myrobolan B on Myrobolan B ..	15	15	100	2,137.1	406.3
Myrobolan B on Common Plum	15	13	87	320.7	40.8
Common Plum on Myrobolan B ..	15	14	93	500.5	45.2
Common Plum on Common Plum	15	14	93	138.3	6.9

* These trees were already budded on Myrobolan B.

TABLE III.

Percentage take and Maiden Growths of Hale's Early Peach and St. Julien Grafts.

Combinations.	No. grafted.	No. take.	% take.	New Wood Growth. cm.	S.E.
Hale's on St. Julien A ..	10	5	50	531.3	69.1
Hale's on St. Julien K ..	10	1	10	85.0	—
St. Julien A on Hale's* ..	20	20	100	656.7	54.7
St. Julien K on Hale's* ..	20	3	15	87.5	22.5
St. Julien A on St. Julien A ..	10	10	100	375.6	45.9
St. Julien A on St. Julien K ..	10	4	40	321.7	63.3
St. Julien K on St. Julien A ..	10	8	80	321.4	25.3
St. Julien K on St. Julien K ..	10	9	90	458.3	116.8

* These trees were already budded on St. Julien A.

TABLE IV.

Percentage take and Maiden Growths of Cherry and Cherry Stock Grafts.

Combinations.	No. grafted.	No. take.	% take.	New Wood Growth. cm.	S.E.
<i>Serrulata</i> on Mazzard ..	20	20	100	166.7	12.7
<i>Serrulata</i> on Mahaleb ..	15	0	0	—	—
<i>Pseudocerasus</i> on Mazzard ..	20	20	100	170.7	20.9
<i>Pseudocerasus</i> on Mahaleb ..	15	4	27	107.7	14.2
Mahaleb on Mahaleb ..	15	15	100	1,982.6	275.1
Mahaleb on Mazzard ..	20	19	95	559.8	51.5
Mazzard on Mahaleb ..	15	15	100	270.9	42.0
Mazzard on Mazzard ..	20	20	100	131.6	14.2

The variety of *Prunus serrulata* used was Ukon; that of *Prunus pseudocerasus* was East Malling F269. The Mazzard used was F5/I, and the Mahaleb was F8/I/10 (both Malling selections).

(b) *Budded trees.* Budding was done in August 1936. All the living buds were counted twice after bud take, once in October 1936 and again in April 1937. The final percentages of bud take and first year's growth are presented in Tables V-VII.

TABLE V.

Percentage Bud take and Maiden Growth of Pears and Quinces.

Combinations.	No. budded.		No. take.	% take.	Wood growth.†
					cm.
Conference on Quince A	..	32	31	97	71.3
Conference on Quince G	..	32	31	97	87.9
Durondeau on Quince A	..	32	31	97	79.4
Durondeau on Quince G	..	32	32	100	31.5
Quince A on Durondeau*	..	30	21	70	—
Quince G on Durondeau*	..	30	18	60	—
Quince A on Quince A	..	32	32	100	844
Quince A on Quince G	..	32	28	88	800
Quince G on Quince A	..	32	27	84	485
Quince G on Quince G	..	32	32	100	432

* These trees were already budded on Quince A.

† Pairs showing insignificant differences in wood growth are bracketed together.

TABLE VI.

Percentage Bud take of Hale's Early Peach and Plum Stocks.

Combinations.	No. budded.		No. take.	% take.
Hale's Early on Brompton	..	24	23	96
Hale's Early on Myrobolan B	..	24	11	46
Brompton on Hale's Early*	..	30	26	87
Myrobolan B on Hale's Early*	..	30	21	70
Brompton on Brompton	..	24	24	100
Brompton on Myrobolan B	..	24	24	100
Myrobolan B on Brompton	..	24	24	100
Myrobolan B on Myrobolan B	..	24	24	100

* These trees were already budded on St. Julien A.

TABLE VII.

Percentage Bud take and Maiden Growth of Plums and Plum Stocks.

Combinations.	No. budded.	No. take.	% take.	Wood growth.*
				cm.
Victoria on Myrobolan B ..	48	44	92	194
Victoria on Common Plum ..	48	44	92	122
Czar on Myrobolan B ..	48	48	100	272
Czar on Common Plum ..	48	17	35	66
President on Myrobolan B ..	48	43	90	342
President on Common Plum ..	48	15	31	44
Myrobolan B on Myrobolan B ..	48	48	100	819
Myrobolan B on Common Plum	48	41	85	417
Common Plum on Myrobolan B	48	47	98	485
Common Plum on Common Plum	48	43	90	208

* In every case the trees on Myrobolan B have made significantly more growth than those on Common Plum.

From the data presented in Tables I-VII, it is clear that the same manifestations of compatibility and incompatibility were displayed in both stock-scion and scion-stock combinations, and examples are illustrated in Figs. 1 and 2. This is contrary to the experience of Chandler (5) with cherries, Heppner and McCallum (24) with plums, and Toxopeus (61) with Citrus.

Among all the combinations tested, there was a somewhat lower percentage take on grafted than on budded trees. Moreover, the difference in percentage take between compatible and incompatible combinations seems much wider among grafted than budded trees, as is clearly shown between Quince A and Quince G on Durondeau and Hale's Early Peach on St. Julien A and St. Julien K. However, there was a clear indication of high percentage of bud failure on very highly incompatible combinations, such as Quince G on Durondeau. In other less acute cases, such as Durondeau on Quince G, budding seems to afford very much less indication of incompatibility, judging from the percentage of bud take; nevertheless, decline in vigour invariably appeared at a later date.

There was a certain amount of variation in the time of appearance of incompatibility and in its degree of severity (or in both), between the scion-stock and the stock-scion combinations in each group. For instance, the symptoms showed up earlier and were more marked in Quince G on Durondeau than in Durondeau on Quince G. Similarly, Common Plum on Czar, showed incompatibility much earlier than Czar on Common Plum, Hale's Early Peach on Myrobolan B than Myrobolan B on Hale's Early Peach, and St. Julien K on Hale's Early Peach than Hale's Early Peach on St. Julien K.

The stock on stock series also showed some degree of incompatibility in those combinations in which the incompatible stock was used as the scion. Thus,

Quince G grafted on Quince A made much less new wood growth in the first year than Quince G on Quince G. On the other hand, the reciprocal grafts of Quince A on Quince G made more growth in the first year than when worked on themselves. In neither case, however, are these indications confirmed by the growth of the budded trees.

Finally, it is an interesting point that although Hale's Early Peach and Myrobolan B are reciprocally incompatible, it was observed that the buds of Myrobolan B on Hale's Early started to develop in the autumn after budding, and reached a length of about 3 cm. Myrobolan B budded on Myrobolan B and on Brompton behaved in the same way. But the buds of Hale's Early Peach on Myrobolan B started to grow at a much later date in the spring of 1937 than did those on Brompton. The same phenomenon was observed on Czar and President plums budded on Myrobolan B and Common Plum respectively. This probably indicates that there is a lack of affinity at the union in incompatible combinations, even in the dormant bud stage just after budding. It is also conceivable that the Myrobolan B, an early and active grower, has retained its potentially early growth somewhat independently of the double worked stock, i.e. of Hale's Early on St. Julien A.

2. *Premature autumn coloration of the leaves, premature flower bud formation, early defoliation and dying back of the young growths.*

All the incompatible combinations in these series showed the general symptoms of premature autumn coloration of the leaves, premature flower bud formation, and early defoliation. In severe cases, the young shoots of the grafts died back early in the season, as shown in Fig. 3. The greater the degree of incompatibility, the earlier the symptoms appeared. Thus, the leaves of the Common Plum grafted on Czar, those of St. Julien K grafted on Hale's Early Peach, of Victoria Plum grafted on Hale's Early Peach and of Quince F and Quince G grafted on Durondeau pear, began to turn yellowish and became markedly mottled as early as the end of June or in July. The leaves of Hale's Early Peach grafted on St. Julien K or Myrobolan B, turned pinkish-red and became curled about the middle of August. Durondeau and Conference pears worked on Quince F and Quince G also showed a yellowish-red colour in the leaves one month earlier than those worked on Quince A.

Whilst it is impossible to secure numerical data concerning these observations, Fig. 3 illustrates well the withering effect and dying back of the young growths and the discoloration of the basal leaves in an incompatible plum combination.

The flower buds of Hale's Early Peach on St. Julien K or on Myrobolan B were prominent the first season after grafting and appeared early in the autumn.



FIG. 1. Hale's Early Peach budded on St. Julien A (left), St. Julien K (centre, incompatible) and St. Julien C (right).
(Photographed : May 26th, 1936.)



FIG. 2. St. Julien C, K, and A (left to right) grafted on Hale's Early Peach. St. Julien K on Hale's Early is incompatible (centre).
(Photographed : May 26th, 1936.)

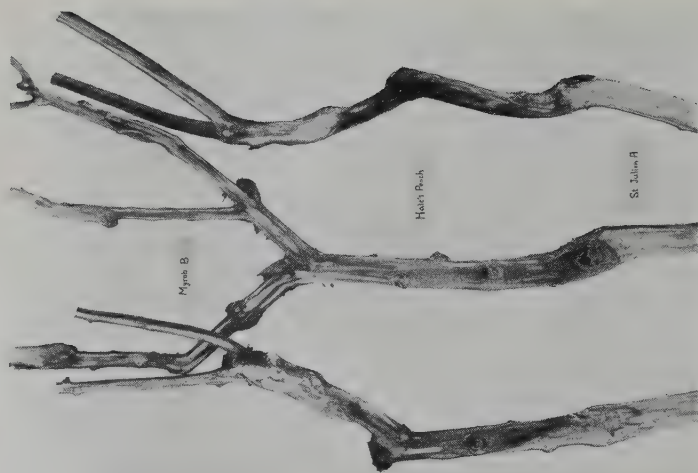


FIG. 4. Gum-formation in Hale's Early Peach stem grafted on St. Julien A, when grafted itself with Myrobalan B.

Note the white healthy wood of the topmost scion—Myrobalan B, and the under stock—St. Julien A. The black tissue of the intermediate Hale's Early is filled with gum.



FIG. 3. Two examples of Common Plum on Czar showing withering and dying back of young shoots.
(Photographed : June 27th, 1936, three months after grafting.)



FIG. 5. Early defoliation of Conference Pear on Quince F (centre).
(Photographed : November 2nd, 1936.)



FIG. 6. Early defoliation of Durondeau Pear on Quince F (left).
(Photographed : November 2nd, 1936.)

With reciprocal grafts of Myrobolan B and Hale's Early, the leaves fell early in the autumn, and by the beginning of the following March all the wood was dry and dead. After examining these dead trees, masses of gum were observed in Hale's Early Peach, whether used as stock or scion, but there were none in the wood of Myrobolan B. This is illustrated in Fig. 4.

The incompatible combinations generally showed earlier defoliation as shown in Table VIII.

TABLE VIII.
Percentage of Defoliation of One-year-Grafts.
(Recorded October 31st, 1936.)

	Combinations.	Condition of Foliage.	% Defoliation.
Pears and Quinces.	Durondeau on Quince A	Dull purplish-green, tip defoliating	30
	Durondeau on Quince G	Ditto	40
	Quince A on Durondeau	Pale yellowish, leathery dry	20
	Quince G on Durondeau	Pale yellowish, dry curling	35
	Quince A on Quince A	Green, still growing	10
	Quince G on Quince G	Ditto	10
Plums and Plum Stocks.	Czar on Myrobolan B	Top leaves green, base defoliating	80
	Czar on Common Plum	Dull green, spotted, tip defoliating	90
	Myrobolan B on Czar	Green, spotted	20
	Common Plum on Czar	Yellowish, dry, tip defoliating	95
	Myrobolan B on Myrobolan B	Green, still growing	10
	Common Plum on Common Plum	Green, just stopped growing	15
Peaches and Plum Stocks.	Hale's on Peach G37/9*	Green, still growing	10
	Hale's on Brompton	Green, lower leaves pinkish	20
	Hale's on Myrobolan B	Few leaves left, reddish, flower buds prominent	99
	Hale's on St. Julien A	Green, lower leaves pinkish	50
	Hale's on St. Julien K	All defoliated, dry wood	100
	St. Julien A on Hale's	Top leaves green, lower leaves yellowish	70
	St. Julien K on Hale's	Yellowish, mottled, dry wood	90
	St. Julien A on St. Julien A	Green, lower leaves yellowish	50
	St. Julien K on St. Julien K	Green, still growing	40

* Peach G37/9, vegetatively raised clonal selection of seedling peach stocks.

Apart from Czar on Myrobolan B and the reciprocal combinations of Hale's Early Peach and St. Julien A (in which early leaf fall is a varietal characteristic) it will be seen that a much greater proportion of the foliage had dropped by October 31st, from the incompatible than from the compatible combinations.

Moreover, the symptoms of earlier defoliation, etc., were more pronounced on the older trees of the second year's growth. Figs. 5 and 6 show the uniformly defoliating trees of Conference and Durondeau pears worked on three selections of quince stocks, and the earlier defoliation is very obvious with Conference and Durondeau on Quince F stocks.

Finally, it was observed that in all incompatible combinations, the leaves at the top of the branch fell first, whereas in all compatible combinations, these

leaves usually remained attached for a long period after the lower leaves had fallen.

3. *Gradual Decline of Annual Wood Growth.*

A comparative study was made of the annual wood growth of compatible and incompatible combinations. The trees were planted in replicated plots and had been uniformly treated since planting. All the trees in each plot were measured and averaged and the results are given in Tables IX-XI.

TABLE IX.
Annual Wood Growth. Pears on Quinces.

Combinations.	Number and age of trees measured.					
	26-39 one-year trees.		29-40 two-year trees.		10 three-year trees.	
	cm.	S.E.	cm.	S.E.	cm.	S.E.
Conference on Quince A ..	69	2.9	407	12.8	334	14.3
Conference on Quince C ..	81	2.8	348	9.4	—	—
Conference on Quince F ..	75	3.0	385	11.6	216	18.0
Durondeau on Quince A ..	79	3.0	559	20.7	529	37.4
Durondeau on Quince C ..	94	2.9	561	21.1	—	—
Durondeau on Quince F ..	50	3.0	117	6.9	117	14.3

TABLE X.
Annual Wood Growth. Plums on Plum Stocks.

Combinations.	Number and age of trees measured.					
	25-39 one-year trees.		33-39 two-year trees.			
	cm.	S.E.	cm.	S.E.	cm.	S.E.
Victoria on Myrobalan B	223	6.3	714	18.0		
Victoria on Common Plum	173	5.7	444	19.4		
Czar on Myrobalan B	219	7.1	836	24.4		
Czar on Common Plum	88	6.6	254	23.6		

TABLE XI.
Annual Wood Growth. Peaches on Plum Stocks.

Combinations.	6-19 one-year trees.	
	cm.	S.E.
Hale's Early on Peach G37/5	1,543	227.9
Hale's Early on Peach G37/9	1,025	135.1
Hale's Early on Brompton	898	86.8
Hale's Early on Myrobalan B	187	26.0
Hale's Early on St. Julien A	836	40.4
Hale's Early on St. Julien C	678	27.1
Hale's Early on St. Julien K	46	15.4

Among the pears on quince stocks, Conference showed more new wood growth on Quince F than on Quince A in the first year, but there was a gradual decrease in the second and third years. On the other hand, Durondeau made slightly less growth in the first year on Quince F than on Quince A, and showed a very rapid decline in the second and third years.

Among the plums, Czar had made much less wood growth by the end of the first season on Common Plum than on Myrobalan B. In the second year the difference was even more pronounced, the incompatible combination producing

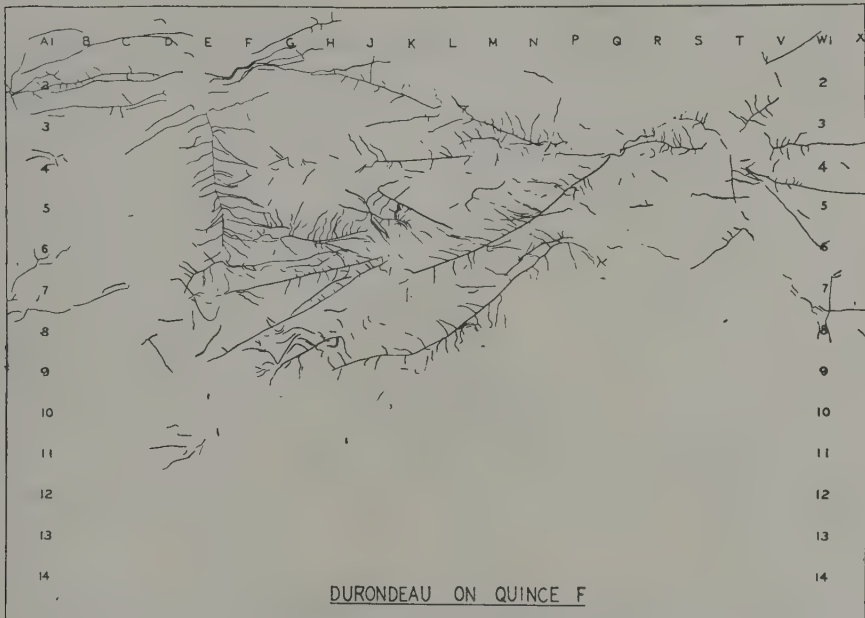


FIG. 7. Pear—Durondeau on Quince F. Showing extension and depth of new root growths as observed in Root Observation Box—1936.

only one-quarter the length of wood formed by the compatible one. Victoria had made slightly less growth in the first year on Common Plum than on Myrobalan B, but the difference in the second year was in about the same proportion. This was probably due to the normal influence of the Common Plum as a dwarfing stock.

With peaches, one-year-old trees only were available, but the difference in wood growth was very great. Hale's Early had made less than one-twentieth of the amount of growth on St. Julien K than on St. Julien A. It had made only about one-fifth of the amount of growth on Myrobalan B that it made on

Brompton. Hale's Early on Brompton and on St. Julien A made about equal amounts. Hale's Early on peach stock G37/9 made the greatest amount of wood during the end of the first season, and from the appearance of the foliage, it looked the healthiest of all the combinations.

As with the symptoms previously described, the more incompatible the combination the more rapid the decline in the annual wood growth.

An interesting phenomenon was observed in this connection, namely that all the incompatible combinations actually made more wood growth than the

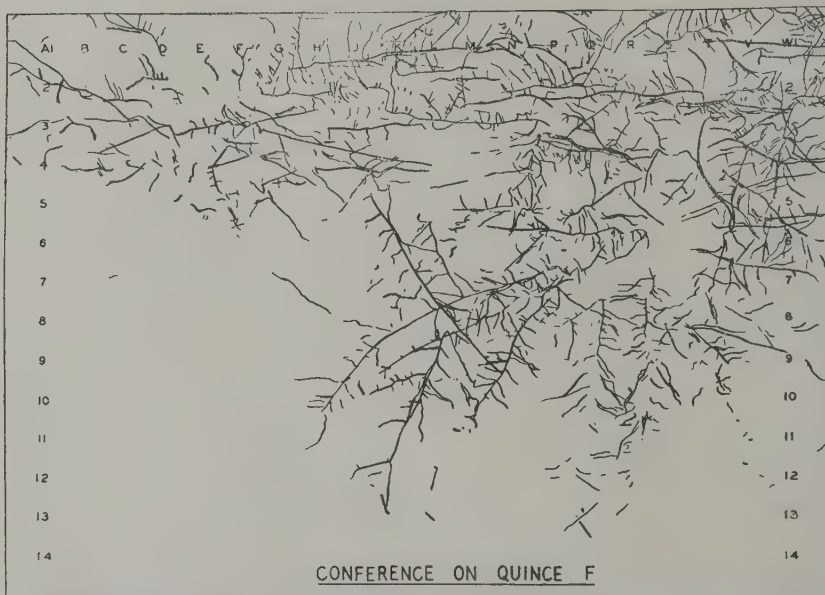


FIG. 8. Pear—Conference on Quince F. Showing extension and depth of new root growths as observed in Root Observation Box—1936.

compatible combinations in the initial stage. In all cases, however, it was followed by a subsequent decline. Tukey (62) observed the same thing with *Pyrus communis* varieties worked on *P. serotina*, *P. calleryana* and *P. ussuriensis*. This point will come out more clearly in the section dealing with scion/stock ratio.

4. Gradual decline of new root growth.

Observations and measurements of new root growth were made by the root observation box method designed by Rogers (53). Two-year-old trees

approximately equal in size were planted in root observation boxes with windows on their north and south sides. The soil (half fine quartz sand and half loam) was mixed and steam-sterilized before being put into the boxes. The trees were root pruned uniformly, weighed, and planted in November 1935.

Owing to the limited number of observation boxes and the enormous amount of labour entailed in recording new root growths, only two scion varieties and two stocks in each group were chosen. However, the arrangement of planting was designed to facilitate observation of one variety on a compatible stock

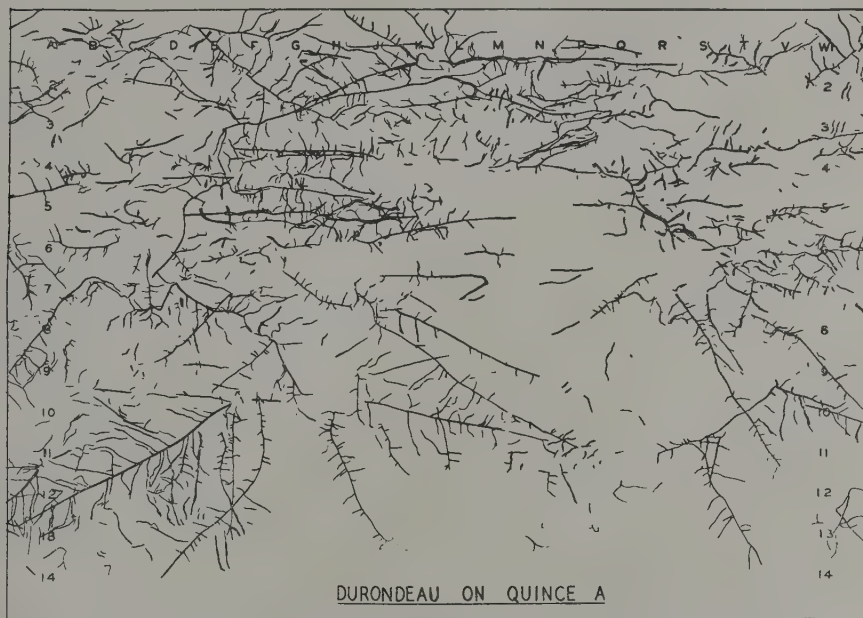


FIG. 9. Pear—Durondeau on Quince A. Showing extension and depth of new root growths as observed in Root Observation Box—1936.

(e.g. Durondeau on Quince A) and the same variety on an incompatible stock (e.g. Durondeau on Quince F). It was also possible to make comparisons with another combination which is supposed to have a much delayed occurrence of incompatibility, such as Conference on Quince F. With this arrangement it was therefore possible to observe the growth of the same scion variety on compatible and incompatible stocks, and also the root growths of the same stock variety carrying two different scion varieties.

Only one variety of peach, Hale's Early, was tested on compatible and incompatible St. Julien selections. In order to ascertain whether there was any

difference in the vigour and period of root growths between these two St. Julien selections, unworked stocks were planted side by side in the box separated by glass in the middle, and the new root growths of each were recorded and compared.

Two trees of one of the following combinations were planted in each root box :

- Conference on Quince F.
- Durondeau on Quince F.
- Durondeau on Quince A.
- Czar on Myrobolan B.
- Czar on Common Plum.
- Victoria on Common Plum.
- Hale's Early Peach on St. Julien A.
- Hale's Early Peach on St. Julien K.
- St. Julien A (unworked).
- St. Julien K (unworked).

In order to hasten the symptoms of incompatibility no fertilizers were applied, but equal amounts of water were given during very dry days in the summer.

All new root growths which appeared through the glass were carefully drawn every fortnight on root charts. Up to the time of cessation of root growth in the winter of 1936 the increments in length of the already visible roots and the lengths of new roots developed during each fortnightly period were recorded and added together. Three of the actual root charts of pears on quince stocks are reproduced in Figs. 7, 8 and 9.

Table XII shows the total number of new roots and the total lengths of new root growth for each combination of two trees from two windows as recorded from April 7th to November 3rd, 1936.

TABLE XII.
New Root Growth of Pear, Plum and Peach Combinations.

Combinations.	Total No. of New Roots.		Total Lengths (cm.).
Durondeau on Quince A	4,160		14,755
Durondeau on Quince F	1,812		6,999
Conference on Quince F	3,335		14,282
Czar on Myrobolan B	3,727		16,842
Czar on Common Plum	2,383		9,082
Victoria on Common Plum	2,694		11,384
Hale's Early on St. Julien A	1,662		5,956
Hale's Early on St. Julien K	12		36
St. Julien A (unworked)*	1,207		5,106
St. Julien K (unworked)*	1,612		6,509

* Only one tree planted in each box.

It will be seen that the incompatible trees had a smaller number and a smaller total length of new roots than the compatibles. For instance, the compatible combination Durondeau on Quince A produced more than double the total length of new roots in one season's growth than the incompatible combination, Durondeau on Quince F. Likewise, Conference on Quince F (a much delayed incompatible combination) had developed more than double the total length of new roots in one season as compared with the more acute form of incompatible combination, Durondeau on Quince F. This fact probably indicates that the symptoms of incompatibility proceeded from the scion downwards. The greater the degree of incompatibility, the more rapid and more marked was the decline in root growth.

The root charts show that the extension of the new root growths of the incompatible combinations was very much limited both horizontally and vertically. For example, the new roots of Durondeau on Quince F and of Czar on Common Plum, were much shallower and more sparse than those on the corresponding compatible combinations.

Another very interesting point was that the first appearances of new root growths from the incompatible combinations occurred much later in the spring than those from the compatible combinations. The new roots of Durondeau on Quince F started to show themselves about six weeks later than those of Durondeau on Quince A. The former combination was also much later than the delayed incompatible combination Conference on Quince F. Hale's on St. Julien A started about four weeks later than St. Julien A unworked. The same phenomenon was observed again in the same boxes in the Spring of 1937. The root growth curves for pears and plums, recorded fortnightly, show the delayed growth of new roots on incompatible combinations (see Figs. 10 and 11).

Furthermore, it was noted that although St. Julien A and St. Julien K represent two extremes of compatibility and incompatibility for Hale's Early Peach, yet (as can be seen in Table XII) there was no significant difference on unworked stocks either in the number or the total length of the new roots.

The dates of termination of the new root growths for compatible and incompatible combinations were also compared. It was quite obvious that Durondeau on Quince F ceased to grow later in the season than Durondeau on Quince A. It would appear that these differences in the periods of starting and terminating root growth cannot be explained by scion influence alone, but that they are likely to be due to some form of incompatibility at the union.

It is often asked whether in an incompatible combination, the scion or the root dies first. By using this root box method it is hoped that some light may be shed on this problem. In the more severe cases of incompatibility (Durondeau on Quince F) root growth had decreased very considerably, whilst in the less acute case (Conference on Quince F) about the same amount of new root growth

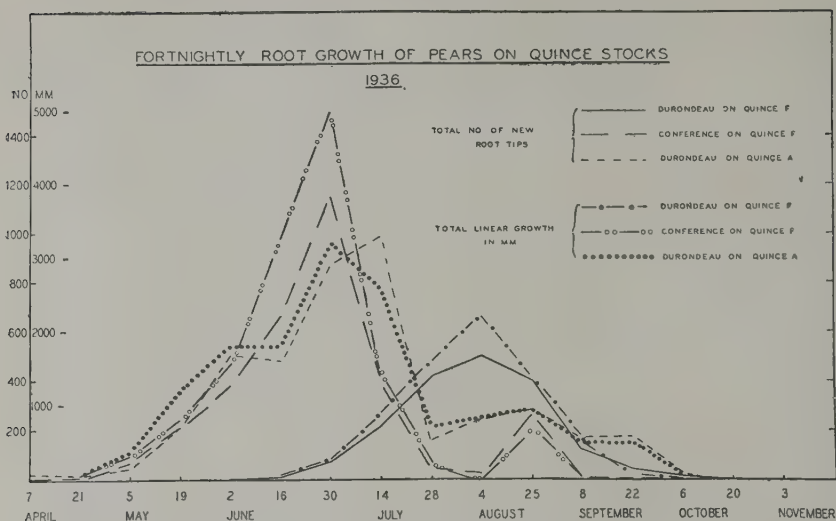


FIG. 10. Fortnightly Root Growth of Pears on Quince Stocks. Note the slow growth of the incompatible combination Durondeau on Quince F.

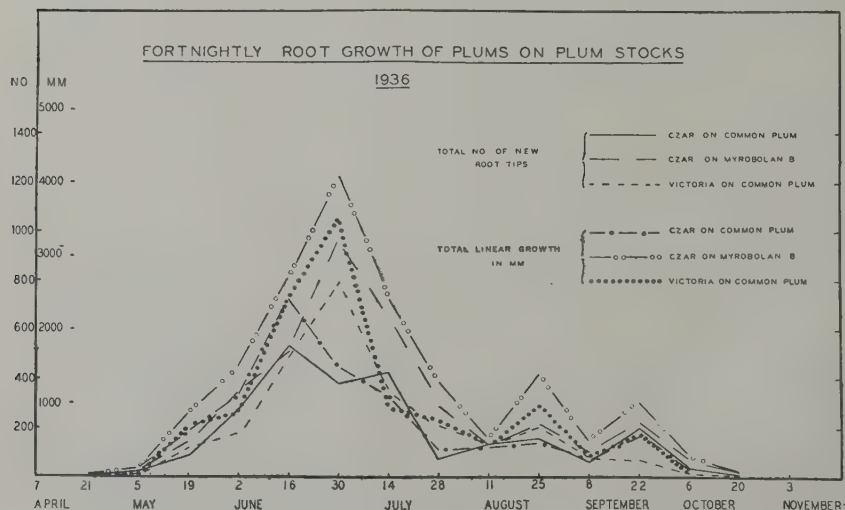


FIG. 11. Fortnightly Root Growth of Czar and Victoria Plums on Plum Stocks. Note the slow growth of the incompatible combination Czar on Common Plum.

had been maintained as in the compatible combination Durondeau on Quince A, although the new shoot growth of Conference on Quince F had decreased quite considerably at the time of recording. Moreover, observations in the field showed that from many incompatible combinations a strong bunch of suckers often arose from the stock when the scions were dead. Again, in many cases, the withering and dying back of the scions proceeded from the tops downwards, as previously described. It seems quite reasonable to think, therefore, that the scion declined first.

5. *Scion/stock ratio.*

In order to study more closely the relationship between stock and scion in incompatible combinations, a series of uniformly grown trees was carefully excavated by the trench method described by Rogers (52). The roots were washed as soon as excavated, and the trees were weighed as soon as they had become air-dry. Plans of their root systems were drawn.

Dry weights of pear combinations were also taken. Their scions and stocks were cut into short pieces and dried in an oven for five days at a temperature of 90°-100° C.

The scion/stock ratios obtained for pears, plums and peaches are presented in Tables XIII-XV.

TABLE XIII.

Scion/Stock Ratio for Pears on Quinces. Average dry weight of Ten Two-year-old Trees for each Combination.

Combinations.	Mean Scion weight (gm.).	Mean Stock weight (gm.).	Scion/Stock Ratio.
Conference on Quince A	176	123	1.47
Conference on Quince C	184	164	1.13
Conference on Quince F	183	102	1.80
Durondeau on Quince A	143	118	1.23
Durondeau on Quince C	180	132	1.37
Durondeau on Quince F	33	56	0.58
Pitmaston on Quince A	176	126	1.39
Pitmaston on Quince C	154	126	1.20
Pitmaston on Quince F	183	128	1.43
Gen. Mean = 1.29			
S.E. = 0.06			
Sig. Diff. = 0.18			

With Conference, the scion/stock ratio was largest on Quince F. There were significant differences between all stocks in the order of magnitude of Quince F, A, and C.

With Durondeau, the scion/stock ratio was smallest on Quince F. The ratios for the trees on Quince A and Quince C were not significantly different from each other, but the ratio for that on Quince F was significantly smaller than that for those on Quince A and C.

There was no marked difference with Pitmaston Duchess on the three stocks.

In addition there were significant differences when Conference, Durondeau and Pitmaston on all stocks were compared, and also when the three rootstocks were compared worked with all scions.

The results obtained with plums fully confirm those obtained with pears. Czar worked on the incompatible Common Plum stock had a much lower scion/stock ratio than Czar on the compatible Myrobolan B. Victoria, which grows healthily on both of these stocks, showed the same kind of effect, but to a much less degree. The difference between stocks showed a highly significant effect. There were again significant differences between the two varieties.

TABLE XIV.

Scion/Stock Ratio for Plums on Plum Stocks. Average dry weight of Fifteen Two-year-old Trees for each Combination.

Combinations.	Mean Scion weight (gm.).	Mean Stock weight (gm.).	Scion/Stock Ratio.
Czar on Myrobolan B	1,286	527	2.35
Czar on Common Plum	273	190	1.40
Victoria on Myrobolan B	838	482	1.86
Victoria on Common Plum .. *	455	325	1.40
			Gen. Mean = 1.75
			S.E. = 0.08
			Sig. Diff. = 0.23

Table XV shows that Hale's Early Peach on the incompatible Myrobolan B stock was significantly different in scion/stock ratio from the same variety on three compatible stocks. The differences between G37/5 and G37/9 and Brompton stocks, all compatible with Hale's Early Peach, were not significant.

An initially higher, but subsequently lower, scion/stock ratio was observed both on Hale's Early Peach on Myrobolan B and on the reciprocal combination, but in the former it showed up much earlier in the season. From bud break in the spring until about the middle of July, all the trees of Hale's Early grown on Myrobolan B made much quicker and stronger top growth as compared with those worked on Brompton or on peach stocks G37/5 and G37/9; but after the middle of July, the growth of Hale's Early on Myrobolan B began to drop, with

the occurrence of other symptoms, like drying, curling and premature coloration of the leaves. Durendeu Pear on Quince G, Victoria Plum on Hale's Early Peach, Czar Plum on Common Plum and other incompatible combinations showed the same phenomena. Fig. 12 shows the growth of scion and stock of Durendeu pear on Quince F, A, and C combinations reconstructed after excavation.

III. THE IMMEDIATE CAUSES OF DECLINE AND PREMATURE DEATH IN INCOMPATIBLE COMBINATIONS.

It is fairly common in the nursery for grafted or budded trees of incompatible combinations to break at the union. It is also not uncommon to see big trees in the orchard (after eight or ten years of growth) suddenly blown down by a gale, broken clean across at the union. Such disasters suggest that incompatible combinations are not firmly united. Moreover, the external symptoms of incompatible combinations, such as withering and early defoliation, gradual

TABLE XV.

Scion/Stock Ratio for Peaches on Plum and Peach Stocks. Average dry weight of Seven One-year-old Trees for each Combination.

Combinations.	Mean Scion weight (gm.).	Mean Stock weight (gm.).	Scion/Stock Ratio.
Hale's Early on Peach G37/5	544	430	1.28
Hale's Early on Peach G37/9	393	259	1.18
Hale's Early on Brompton	345	268	1.29
Hale's Early on Myrobolan B	51	89	0.58
			Gen. Mean = 1.08
			S.E. = 0.08
			Sig. Diff. = 0.25

decline in wood and root growth, and the dying back of the scion irrespective of the still living and suckering stock, etc., already described, suggested that there might be some form of obstruction to water or food transport at the union. The following experiments were therefore designed to study in detail these features of the defective union in incompatible combinations, for they may be the immediate causes of the gradual decline and premature death of the tree.

1. *Mechanical weakness of the union.*

Budded and grafted trees were examined in the field and laboratory to ascertain whether the incompatible combinations had any form of abnormality or weakness in the structure at the union.

(a) *Wood and bark discontinuity.* Trees were examined both externally, and with the bark peeled off at the union. Three forms of discontinuity of wood and bark were observed, namely :

- (i) Bark continuous, but wood discontinuous ; observed in Czar on Common Plum.
- (ii) Bark discontinuous but wood continuous ; observed in Durondeau on Quince A.
- (iii) Both bark and wood discontinuous ; observed in Durondeau on Quince F.

Figs. 13 and 14 show clearly these three forms of discontinuity.

In the pear, Dr. Jules Guyot worked on C_2 (a *Pyrus communis* seedling selection), both the bark and wood were very smooth and continuous (Fig. 13). Durondeau on Quince A showed almost smooth bark but a shallow crevice in the outside cortical region, whereas the wood fibres were smooth and continuous. Durondeau on Quince F showed a deep crevice in the bark, and a thick layer of "corky" cells in the wood at the union.

In the plum series, Czar on Myrobolan B showed continuity in bark and wood (Fig. 14), whereas both Czar on Common Plum and Oullin's Golden Gage on Myrobolan B (an exceptional known case of incompatibility) had a very distinctive crevice in the wood, at the union, although this was not externally visible.

These distinctive groups of incompatibility as shown by bark and wood discontinuity would seem to be directly correlated with a high degree of incompatibility. The earliest symptoms of decline appeared in combinations in which both bark and wood were discontinuous. On the other hand, much delayed symptoms of decline occurred in combinations in which only the bark or only the wood showed discontinuity. Possibly this crevice at the union hinders or lessens the upward and downward transport of water, soil solution and manufactured food, thus causing a weaker growth of shoot and root.

(b) *Parenchymatous cell accumulations.* Many strikingly incompatible grafts showed an accumulation of parenchymatous tissue at the union, visible to the naked eye. In order to obtain a more distinctive contrast between stock and scion tissues the purple-tissued species of apple (*Pyrus Niedzwetzkyana* Hemsley) was used, both as stock and scion, in combination with apple, quince and pear varieties. Some of these combinations resulted in unexpectedly vigorous growth. *Pyrus Niedzwetzkyana* on Quince A, for example, formed shoots 3-4 feet long by the end of the season, but when longitudinal cuts were made



FIG. 12. Excavated Pears—Durondeau on Quince F (left), on Quince A (centre), and on Quince C (right), showing root and shoot growth. Two-year-old trees.



Durondeau
on Quince F.

Durondeau
on Quince A.

Dr. G. Jules
on Pear C₂.

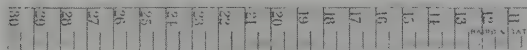


FIG. 13. Bark and Wood Discontinuity—Pears. Durondeau on Quince F (left), both bark and wood discontinuous. Durondeau on Quince A (centre), both bark and wood continuous, with slight crevice at the cortical region inside the bark. Dr. Jules Guyot on Pear C₂ (right) is perfectly continuous.



FIG. 15. Pear, Dr. Jules, Quince A, Apples No. IX and No. XII grafted on *Malus Niedzwetzkyana* stocks. (One-year-old grafts.) Pear Dr. Jules and Quince A (two left) had heavy accumulation of 'corky' cells. Apples No. IX and XII had no accumulation although the colour of tissue remains distinct.



FIG. 14. Bark and Wood Discontinuity—Plums. Czar on Myrob. B has both bark and wood continuous (left). Czar on Com. Plum has a continuous bark but discontinuous wood (right).

through the union, a thick layer of discoloured cells was visible between the purple tissue of the scion and the white tissue of the stock (Fig. 15).

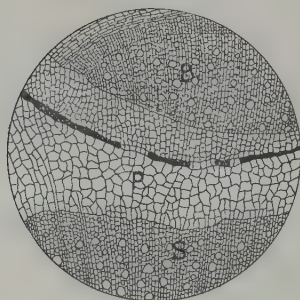
On the other hand, with apple rootstock XII on *P. Niedzwetzkyana* there was a distinct line of demarcation between the white and the purple tissue of scion and stock with no trace of discoloration, although this combination had made slightly less vigorous growth than had *P. Niedzwetzkyana* on Quince A. Fig. 15 shows the demarcation at the union in pear, quince, and apple grafts.

In order to follow the development of this parenchymatous tissue, the anatomy of the unions was studied in some 800 sections of inserted buds before these started to unfold. Plum and quince stocks were planted out in rows in the winter of 1935, on the randomized block system, and were budded in the summer of 1936 at random, within the stock rows, with varieties of plums, peaches, pears, and with the stocks themselves.

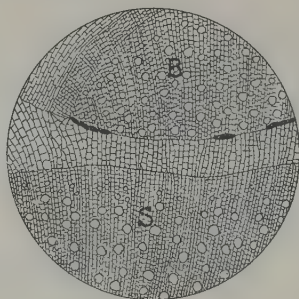
After budding, samples of the inserted buds were taken fortnightly, starting two weeks after insertion and continuing until April 1937. All the portions to be sectioned were fixed at once in Farmer's fixative, and then stored in 70 per cent. alcohol. Transverse sections 20 μ thick were cut on a sliding microtome, and stained with safranin and Delafield's haematoxylin.

In the stock on stock combinations, such as Myrobolan B on Myrobolan B, newly developed cells between the bud and the adjoining stock were first observed about four weeks after budding. In the compatible scion-stock combinations, such as Czar on Myrobolan B, these cells were observed about six to eight weeks after budding, but in the incompatible combinations, such as Czar on Common Plum, the buds adhered very loosely to the stock and consequently were very difficult to cut, even twelve weeks after budding. Instead of growing together more or less uniformly the new cells at the union were much enlarged and were arranged in a markedly irregular way. This large-celled tissue, in which lacunae were sometimes present, was produced either by the stock, as in Hale's Early Peach on Myrobolan B, or by the bud, as in Czar on Common Plum. In later samples these irregular and loosely arranged cells were seen as masses of dead tissue. Both cross and longitudinal sections were examined, and both presented the same feature in compatible unions, viz. large-celled parenchyma and, later, dead cell accumulations as shown in Fig. 16. The pears on quince stocks did not exhibit these phenomena until much later in their course of growth. However, in every incompatible combination, these abnormal cell accumulations increased more and more as the bud growth advanced. In the delayed form of incompatibility these abnormal cells were present to a much less extent in the earlier stages, but as the buds grew the accumulations became larger and more prominent. Fig. 16 shows diagrammatically the contrast in new tissue formation at the union between incompatible and compatible combinations as seen in the course of microscopical examinations.

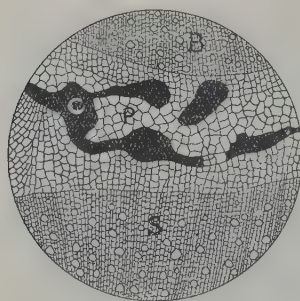
(c) *Ease of breaking at the union and nature of the break.* In order to see whether the presence of large-celled parenchyma and dead cell accumulations



Sampled: September 15th.

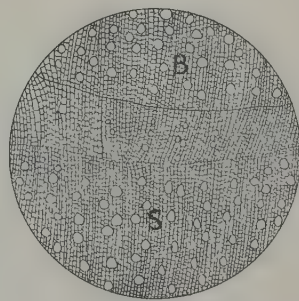


Sampled: September 15th.



Sampled: November 15th.

President on Common Plum.



Sampled: November 15th.

Common Plum on Common Plum.

Fig. 16. Diagrams showing Transverse Sections of Bud-Unions. Plums—Budded August 4th, 1936.

B=Bud.

S=Stock.

P=Enlarged parenchymatous cells.

C="Corky" dead cells.

would lead to mechanical weakness of the union, special apparatus was designed by the author to measure the force required to break grafted trees at the union. This is shown in Fig. 17.

Stems of compatible and incompatible combinations containing the piece of union were fixed and tightly held by strong clamps in the position shown in Fig. 17.

In this figure A is a fixed point. The distance from B to C is ten times the distance from B to A, thus making a tenfold leverage. An upward pull indicated as 1 lb. on the spring balance means a force of 10 lb. at the graft union.

A large spring balance was fastened at the end for pulling and reading. A wire-strainer was used so that before each pull the lever could be adjusted

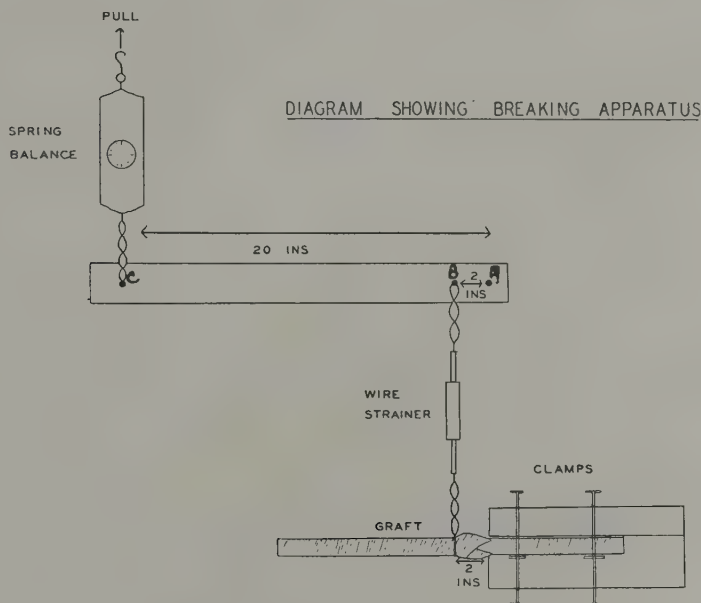


FIG. 17. Diagram of Breaking Apparatus for Strength-at-Union Test.

exactly parallel with the graft union. For the tests, six trees of pear on quince and plum on plum, and seven of peach on plum and peach, of uniform size, of each combination, were used. The tests were made in the Spring of 1937.

It was noted that compatible unions of two-year-old pears, plums and peaches required a pull of as much as 700 lb. to break them.

The number of pounds required to break each union were calculated per square centimetre of the cross-sectional area of the union, and these are given in Tables XVI-XVIII.

All three varieties of pears, Conference, Durondeau and Pitmaston, when worked on Quince A, revealed a very significantly stronger union as compared with those worked on Quince F, except Conference on Quince F, which has a much delayed form of incompatibility. The weakness of the union as expressed by this test seems to be in direct proportion to the degree of incompatibility.

TABLE XVI.

*Strength-at-Union Test of two-year-old Pears on Quince Stocks.**

Combinations.			Mean Diameter at Union (cm.).	Mean total lb. required to break.	Mean lb. to break per sq. cm. of cross-sectional area.
Conference on Quince A	..	..	3.41	199.5	21.7
Conference on Quince C	..	..	3.63	278.3	26.9
Conference on Quince F	..	..	3.53	214.3	21.9
Durondeau on Quince A	..	..	2.88	211.3	32.7
Durondeau on Quince C	..	..	2.84	150.0	23.6
Durondeau on Quince F	..	..	1.63	18.7	9.1
Pitmaston on Quince A	..	..	3.37	214.8	24.1
Pitmaston on Quince C	..	..	2.89	164.2	24.7
Pitmaston on Quince F	..	..	3.48	145.8	15.4

Gen. Mean = 22.2 lb. per sq. cm.

S.E. = 1.23

Sig. Diff. = 3.88

* A further series of twelve trees each of these pear and quince combinations have been tested since the above was written, and yielded exactly the same results.

TABLE XVII.

Strength-at-Union Test of two-year-old Plums on Plum Stocks.

Combinations.			Mean Diameter at Union (cm.).	Mean total lb. required to break.	Mean lb. to break per sq. cm. of cross-sectional area.
Czar on Myrobolan B	..	..	3.91	†685.7	†76.9
Czar on Common Plum	..	..	1.92	168.2	52.7
Victoria on Myrobolan B	..	..	3.33	515.4	59.9
Victoria on Common Plum	..	..	2.30	261.4	62.5

Czar on Myrobolan B : Czar on Common Plum.

S.E. difference between means = 8.8

Significant Difference = 18.9

Victoria on Myrobolan B : Victoria on Common Plum.

S.E. difference between means = 5.8

Significant Difference = 12.5

† Bent, but still unbroken.

TABLE XVIII.

Strength-at-Union Test of two-year-old Peaches on Plum and Peach Stocks.

Combinations.		Mean Diameter at Union (cm.).	Mean total lb. required to break.	Mean lb. to break per sq. cm. of cross-sectional area.
Hale's Early on Peach G37/5	..	2.64	393.7	73.7
Hale's Early on Brompton	..	2.47	302.3	63.1
Hale's Early on Myrobolan B	..	1.57	54.0	25.6

Hale's Early on Peach G37/5 : Hale's Early on Brompton.				
			S.E. difference between means =	9.2
			Significant Difference =	19.7
Hale's Early on Brompton : Hale's Early on Myrobolan B.				
			S.E. difference between means =	3.9
			Significant Difference =	8.7

These three scion varieties when worked on Quince C gave rather variable results. The trees of Conference on Quince C had a comparatively strong union, whereas these of Durondeau and Pitmaston on Quince C had a somewhat less strong one. Moreover, among the same group of trees tested, such as Durondeau on Quince C, two trees out of six showed a relatively easy and clean break, whereas the four others showed fairly difficult and rough breaks. Pitmaston Duchess on Quince C also behaved in just the same way. The same thing was observed when another series, of twelve trees each, was tested in July 1937. This indicates that Quince C is probably a partially incompatible stock for Durondeau and Pitmaston Duchess pears, though this incompatibility is often indefinitely delayed.

With plums, most of the unions in trees of Czar on Myrobolan B could not be broken at pull of 700 lb. and they are very significantly stronger than the unions of Czar on Common Plum. There was no significant difference between the unions of Victoria on Myrobolan B and on Common Plum.

Again, with peaches, the incompatible Hale's Early had a very much weaker union on Myrobolan B than on Brompton. The difference between Hale's Early on Peach G37/5 and on Brompton is not significant.

It was noted that the force required to break the unions between Pitmaston Duchess pear and Quince F stock was much less than that necessary to break the unions between the same pear scion and Quince stocks A and C; although, as was shown in the scion/stock ratio study, this variety had made much more vigorous growth on Quince F than on either of the compatible stocks at the time of the breaking-test.

Furthermore, an examination of the broken surface of the incompatible unions, such as Durondeau on Quince F or Czar on Common Plum, revealed the presence of inwardly curved layers of cells produced by the stock and the scion,

such as occur in the healing of wounds. This suggests that the union between the stock and scion formed in successive seasons had been incomplete.

It is obvious, therefore, that all incompatible combinations possessed relatively weak unions. Moreover, in every case tested, they had invariably a clean and smooth break, as is clearly shown in Durondeau on Quince F in Fig. 18. The same phenomenon was observed with Czar on Common Plum and Hale's Early Peach on Myrobalan B. On the other hand, all the compatible unions showed a very rough and irregular break. These differences are also shown in Fig. 18.

2. Obstruction of water, soil nutrients and elaborated foods at the union.

The symptoms of withering and premature autumn coloration of the foliage, together with those of premature flower bud formation and early defoliation, as already described, suggest that there may be a deficiency of moisture content in the scion due to obstruction at the union. To determine whether lack of water existed in incompatible combinations above the union, the following experiment was carried out to show statistically the moisture content of the leaves and shoots.

Samples of leaves and current-year shoots were taken from compatible and incompatible combinations on September 18th, 1936. Four samples were taken from four different trees of each combination. The petioles were removed from the leaves before weighing. Samples were also taken from unworked stocks to ascertain whether any difference existed between the so-called compatible and incompatible stocks. Immediately after the shoots were sampled in the field, the fresh weights of the shoots and leaves were determined. Then, all samples were put into an electric oven to dry for three days at a temperature of 90°-100° C. Dry weights were then determined and the moisture content of the different samples was calculated. The results are given in Tables XIX, XX and XXI.

TABLE XIX.

Moisture Content of Pears on Quinces.

Combinations.		% of moisture of one-year trees.				% of moisture of two-year trees.			
		Stems.		Leaves.		Stems.		Leaves.	
			S.E.		S.E.		S.E.		S.E.
Durondeau on Quince A	..	55.8	1.1	60.4	0.2	51.5	0.4	57.6	0.4
Durondeau on Quince F	..	50.8	0.5	55.9	0.8	43.7	0.2	48.5	0.4
Conference on Quince A	..	56.1	0.5	62.8	0.3	54.5	0.3	56.6	0.2
Conference on Quince F	..	51.4	0.4	57.8	0.4	50.9	0.3	57.2	0.3
Quince A (unworked)	..	58.4	0.2	61.1	0.1	—	—	—	—
Quince F (unworked)	..	60.2	0.4	61.9	0.3	—	—	—	—

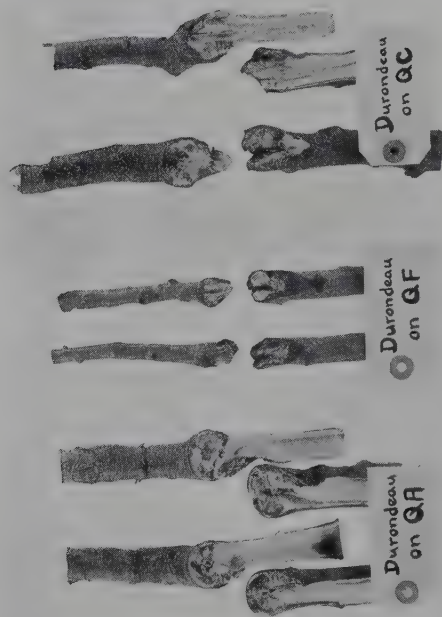


FIG. 18. Strength-at-Union Test—Durondeau on Quince A, F, and C. Durondeau on Quince F (centre) shows a very clean and smooth fracture. Durondeau on Quince C (right) had a few trees with a clean but most of them with a rough fracture. Durondeau on Quince A (left) had a rough, tearing break.

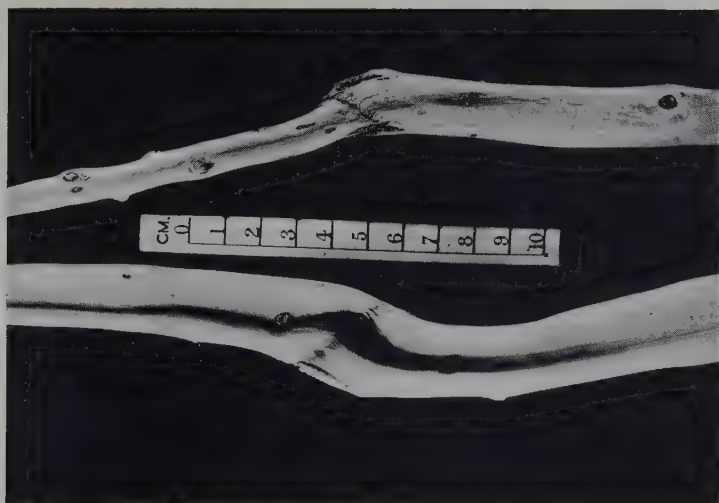


FIG. 19. Dye Injection of Pears. Durondeau on Quince A (left), Durondeau on Quince F (right). Injected July 1936, with 0.2 per cent. Alkali Blue.

TABLE XX.

Moisture Content of Plums on Plum Stocks.

Combinations.	% of moisture of two-year trees.		
	Stems.	Leaves.	
	S.E.	S.E.	
Czar on Myrobalan B	58.5	70.6	0.2
Czar on Common Plum	55.0	69.1	0.4
Victoria on Myrobalan B	53.0	67.9	0.6
Victoria on Common Plum	51.9	69.8	0.3
Myrobalan B (unworked)	60.1	67.0	0.7
Common Plum (unworked)	58.5	66.8	0.7

TABLE XXI.

Moisture Content of Peaches on Plum Stocks.

Combinations.	% of moisture of one-year trees.		
	Stems.	Leaves.	
	S.E.	S.E.	
Hale's Early on Peach G37/5	61.2	70.4	0.2
Hale's Early on Brompton	62.5	69.9	0.5
Hale's Early on Myrobalan B	57.2	58.5	0.4
Hale's Early on St. Julien A	63.7	69.5	0.6
Hale's Early on St. Julien K	45.0	55.2	0.2
Peach G37/5 (unworked)	62.5	71.9	0.3
Brompton (unworked)	64.7	70.8	0.2
Myrobalan B (unworked)	60.2	67.0	0.7
St. Julien A (unworked)	55.6	68.7	0.1
St. Julien K (unworked)	55.5	70.3	0.5

In the same comparable group, Durondeau on Quince F and Conference on Quince F were much lower in moisture content of the shoots and leaves than were the same varieties on Quince A, except in one case of leaves of the second-year-Conference on Quince F. The shoots and leaves of Czar on Common Plum were also drier (especially the shoots) than those of Czar on Myrobalan B, whereas those of Victoria showed approximately the same moisture content on both stocks. Hale's Early Peach worked on St. Julien K and on Myrobalan B, two incompatible stocks for peaches, also had a much lower percentage of moisture in the shoots and leaves than did the same variety worked on Peach seedling selection G37/5, Brompton and St. Julien A, three compatible stocks for peaches.

There was very little difference in moisture content of the unworked stocks. St. Julien A and St. Julien K showed a slightly lower moisture content in the

shoots than the other unworked stocks, but the moisture content of the leaves was about the same.

The phenomenon, like the other symptoms noted above, was most marked in the more incompatible combinations, such as Hale's Early Peach on St. Julien K and Myrobolan B.

In the pear combinations, it was observed that the difference in moisture content between compatible and incompatible combinations increased in direct relation to the age of the tree, except in the leaves of Conference on Quince F, where a slight increase over Conference on Quince A was recorded. This difference may have been due in part to variations in the kinds of tissue in the samples tested. It should be noted, however, that there was practically no difference in moisture content between unworked stocks, especially in the leaves, in spite of the variation in size and morphology of the leaves and stems of those stocks.

The samples were taken at a time when the transpiration rate was high and the trees were still fairly active in growth. It is therefore reasonable to suppose that these differences in moisture content in incompatible combinations may indicate either a partial obstruction to the passage of moisture at the union, or a decline of the root absorbing surface.

In order to determine whether there is any definite form of obstruction to the passage of water or soil solutions or to the transference of food reserves from one partner to the other at the union, a further series of experiments was carried out as follows :

(a) *Dye Injection to show obstruction at the union.* Dyes were injected into the stems of growing trees in July 1936. Small holes were bored with a special drill to about $\frac{1}{4}$ inch in depth both 2 inches above and 2 inches below the union. The portion of the bark at the union was peeled off to observe the ascending and descending flow of dyes. The dyes used were Alkali Blue (0.2%), Water Blue (0.2%), Ponceau Red (0.2%), and Acid Fuchsin (0.2%).

The passage of the dyes was traced after injection to see how long they took to pass the union. It was discovered in preliminary tests that Acid Fuchsin and Ponceau Red travelled too quickly to make accurate measurements possible, Water Blue and Alkali Blue were therefore used in the comparable series. Alkali Blue travelled much more slowly than Water Blue. The dyes were injected by connecting glass tubes with small rubber ones as suggested by Roach (51).

At that time of the year it was found that the upward movement of the dyes was much more rapid and also much greater in volume than the downward movement.

The average results of the Alkali Blue injections of three two-year-old trees each are given in Table XXII.

TABLE XXII.

Results of Alkali Blue Injections in Pears and Plums.

Combinations.				Time required for dye to pass the union after injection.	Amount of dye absorbed 5 hours after injection.
Durondeau on Quince A	..	..	..	5 hours	20 c.c.
Durondeau on Quince F	..	..	..	18 "	6 "
Czar on Myrobolan B	..	..	..	14 "	28 "
Czar on Common Plum	..	..	..	24 "	15 "

Water Blue injections showed similar variation between the compatible and incompatible combinations, but the liquid was absorbed at a much more rapid rate and in greater quantity. This is probably due to the fact that Water Blue is a di- or tri-sulphonic Blue and is more soluble than the mono-sulphonic Alkali Blue. With plums there was practically no difference between Czar on Myrobolan B and Czar on Common Plum in the rate of ascending flow of Water Blue solution.

In incompatible combinations of pears there was a very limited flow of dyes and their paths were much contorted after passing through the union. This indicates that the wood vessels just above the union were contorted and obstructed. On the other hand, Durondeau on Quince A had a very copious and free flow of dye through the union, as shown in Fig. 19.

(b) *Water Conductivity Test to show obstruction at the union.* It had been observed that the incompatible grafts had much smaller leaves as compared with the compatible combinations. This smaller leaf-area persisted every season after grafting. On many highly incompatible grafts the young shoots and leaves died back, usually in the hot summer days of July and August, as shown in Fig. 3. Moreover, the moisture-content determinations of the leaves and young shoots, previously described, showed that there is a definite deficiency of water in the scion leaves and shoots of the incompatible combinations. Hence an experiment on water conductivity through the union was designed, to ascertain whether there was any obstruction at the union to the upward flow of water.

Water conductivity in branches of trees has been studied by Farmer (11), Rivett (49), and many others. Farmer showed that there was great variation between evergreen and deciduous trees, although the variability in each case was very high. Further, Langdon (37) and Holmes (26), described a kind of correlation between wood structure and water conductivity, but the method they employed, involving positive pressure and counting the drops, was too slow to be used on grafts. A negative pressure apparatus was therefore devised by

the author which permits the testing of six grafts at a time. Readings can be made at intervals on six burettes connected to the six grafts, as shown in Fig. 20.

The pear and plum trees used in the experiment were two years old and the peaches one year old. These were freshly dug, and in order to avoid the entrance of excessive air into the vessels, the parts containing the unions were cut under water into 8 cm. lengths (4 cm. above and 4 cm. below the middle of the union). A negative pressure of 735 μ m. of mercury was maintained throughout the experiment with a $\frac{1}{4}$ horse power motor. Fifteen minutes after the motor

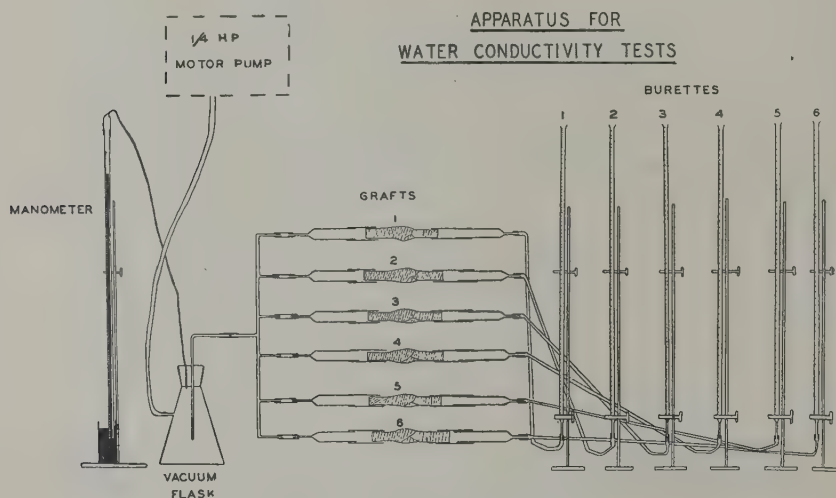


FIG. 20. Diagram of Apparatus for Water Conductivity Test.

started, readings were begun and they were repeated at intervals of ten minutes for one hour.

The total amount of water that passed through the union was calculated in cubic centimetres per sq. cm. of the cross-sectional area, by taking the average of the diameters of the scion and stock, measured 1 cm. above and below the union. Tables XXIII, XXIV and XXV show the results of the experiment, the figures being the average of six trees for each combination.

In every case the incompatible combinations showed a much lower efficiency in conductivity. Durondeau on Quince F passed only one-third of the amount of water that Durondeau on Quince A passed. Czar on Common Plum passed only about one-tenth of the amount of water that Czar on Myrobalan B passed.

TABLE XXIII.

*Water Conductivity Test through the Union—Pears on Quince Stocks.*ERRATUM

Vol. XV, Part 4, page 298, line 7,
for 735 cm. read 735 mm.

	Mean cross section area (sq. cm.).	Mean total water passed in 1 hour (c.c.).	Mean c.c. of water passed per hour per sq. cm.
	8.60	313.9	37.3
	7.56	305.6	40.4
	7.35	328.5	44.5
	5.02	133.5	25.4
Durondeau on Quince C	4.06	109.1	26.8
Durondeau on Quince F	2.97	26.2	8.8
Pitmaston on Quince A	5.74	335.9	58.1
Pitmaston on Quince C	4.61	134.9	28.1
Pitmaston on Quince F	6.11	157.0	25.0
Gen. Mean = 32.7 c.c. per hour per sq. cm.			
S.E. = 2.01			
Sig. Diff. = 6.32			

TABLE XXIV.

Water Conductivity Test through the Union—Plums on Plum Stocks.

Combinations.	Mean cross section area (sq. cm.).	Mean total water passed in 1 hour (c.c.).	Mean c.c. of water passed per hour per sq. cm.
Czar on Myrobolan B	6.68	734.6	113.8
Czar on Common Plum	3.32	30.6	9.2
Victoria on Myrobolan B	6.43	703.9	109.3
Victoria on Common Plum	3.84	283.9	76.2
Gen. Mean = 76.2 c.c. per hour per sq. cm.			
S.E. = 5.12			
Sig. Diff. = 15.0			

TABLE XXV.

Water Conductivity Test through the Union—Peaches on Plum and Peach Stocks.

Combinations.	Mean cross section area (sq. cm.).	Mean total water passed in 1 hour (c.c.).	Mean c.c. of water passed per hour per sq. cm.
Hale's Early on Peach G37/9	2.80	159.1	53.0
Hale's Early on Brompton	4.08	67.7	16.1
Hale's Early on Myrobolan B	1.64	4.3	2.6
Gen. Mean = 23.9 c.c. per hour per sq. cm.			
S.E. = 7.12			
Sig. Diff. = 22.4			

The variation between Hale's Early Peach on Myrobolan B and on Peach G37/9 is even more striking.

Pieces of scion stems taken just above the unions and cut into 8 cm. lengths were also tested in a similar way. It was observed that with pears and plums there was practically no variation between scions that had been worked on compatible and incompatible stocks. Hale's Early Peach on Myrobolan B passed a much smaller amount of water than did Hale's Early on Peach G37/9. After splitting the pieces of scion it was actually found that there were many gum deposits in the vessels of the Hale's scion worked on the Myrobolan B stock. It seems likely that this gum materially lowered the conductivity.

On the budded trees most of the stock stems were too short to allow of a conductivity test. However, 8 cm. stem pieces of the unworked two-year stocks were tested in a similar way, and no appreciable difference was observed between comparable groups.

In the data obtained on the conductivity of the unions, the variability among the six individual trees tested was rather high (Tables XXIII-XXV), but in considering the difference between compatible and incompatible combinations, the figures are still highly significant.

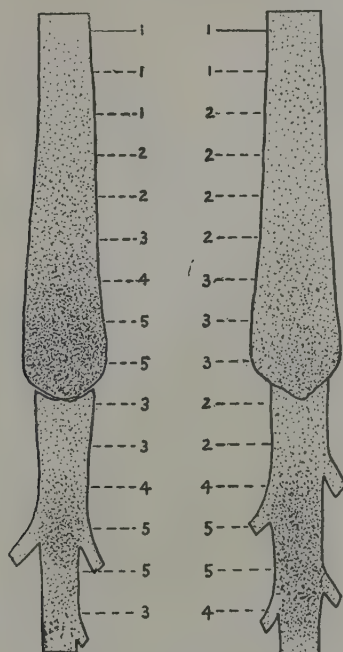
(c) *Examination at the union for starch and for mineral obstruction.* Experiments were made both on young incompatible scions, and on prematurely yellowing leaves by injecting nutrient solutions. The method used was that described by Roach (50), and the following nutrient solutions and their combinations were injected :

1.	Urea, $\text{CO}(\text{NH}_2)_2$	..	..	..	0.5%
2.	Fe-citrate	..	..	..	0.05%
3.	K_2HPO_4	..	..	..	0.5%
4.	K_2SO_4	..	..	..	0.5%
5.	H_2O	..	..	..	control.

Injectations by means of special tubes into young scions which showed the adverse effects due to incompatibility were made in the middle of July 1936 with St. Julien K on Hale's, Common Plum on Czar, and Quince G on Durondeau. No indications of recovery were obtained after injection. It was, however, possibly too late to have injected, as the scions were already showing severe symptoms of decline.

Leaf injections through small rubber tubes were made into ailing pear and peach leaves at 11 a.m. on July 8th and 9th, 1936. Peach leaves were somewhat too thin to hold the rubber tubes and these dropped off overnight. The yellow leaves of Durondeau on Quince F, however, showed a clear recovery, becoming dark green within seven days after injection with 0.05% ferric citrate.

Tests were also made in November 1935 on compatible and incompatible combinations to ascertain the variations in the amount of starch deposited above and below the union. Longitudinal sections 30μ thick were cut at 1 inch intervals along the stem, above and below the union of three Durondeau trees on Quince F and three Durondeau on Quince A. All the trees were two years old. The sections were then put into 0.5% iodine in potassium iodide solution.



Dur. on Quince F. Dur. on Quince A.

FIG. 21. Diagram showing distribution of starch at the Union of Pears. (Two-year-old trees, November, 1935.) The figures 1-5 represent degrees of intensity of starch reaction at distances 1 inch apart.

There was a heavy deposit of starch just above the union in incompatible Durondeau on Quince F as indicated by the iodine test, whereas the portion of stem just below the union had very little starch. The compatible Durondeau on Quince A had a uniformly distributed amount of starch both above and below the union. This is illustrated in Fig. 21, which is a diagram prepared after assembling in order the 1 inch lengths that had been treated with iodine.

This observation appears to be in close agreement with observations made by Kostoff on grafts of certain Solanaceae (34, 35). The starch deposits above the union may possibly be an after-effect of the lack of water in the tissue or may be associated with the early maturity of the incompatible tree; but in considering the starch reaction just below the union, it is clear that there must have been some obstruction to the translocation of sugars and other soluble carbohydrates at the union.

IV. POSSIBLE FUNDAMENTAL CAUSES OF INCOMPATIBILITY.

A common hypothesis held by many workers (14, 15, 16, 61) has been that the fundamental cause of incompatibility is the production of some form of toxin by one of the partners in a grafted or budded tree. However, amongst the deciduous fruit trees studied at East Malling, there were considerable differences in the occurrence and the degree of severity of incompatibility between a normal scion-stock and its reciprocal stock-scion combination. Moreover, Czar, a plum closely related to *P. domestica*, fails to grow on Common Plum (another *P. domestica* stock) whereas it grows very vigorously on Myrobolan B, which belongs to different species of *Prunus*, viz. *P. cerasifera*. Again, Oullin's Golden Gage, another variety of *P. domestica*, fails to grow at all on Myrobolan B, which is generally compatible with other plums. These observations seem to suggest that incompatibility is not likely to be due to the presence of toxic substances such as hydrogen cyanide or to poisonous alkaloids.

On the other hand, observations on the growth of different stocks in the field have often revealed great differences either in the period or in the amount of growth. For instance, Victoria Plum has a much shorter period of seasonal growth than Czar Plum. Likewise, unworked Common Plum stocks have a much shorter period of growth than Myrobolan B. These facts suggest strongly that the cause of incompatibility might be associated with some functional physiological differences in cambial activity, callusing, and other growth activities. Accordingly the following experiments were carried out to determine what effect, if any, the following physiological differences between stock and scion might have upon their compatibility.

1. *Different periods of cambial activity between stock and scion.*

Priestley (41) has shown that different plants vary in their seasonal periods of cambial activity, and has also described a stripping method (42) for studying the period of cambial activity. In accordance with this method strippings were made in the summer of 1936 on unworked stocks generally recognized as compatible and incompatible in each group and upon certain representative scion varieties. All the trees were growing in the same plot. A rectangular cut,

about 1 inch long and $\frac{1}{2}$ inch wide, was made in the stock trunk. The bark was carefully peeled off and the strippings were collected.

It was observed that there was a difference in the stiffness of the bark while peeling at the beginning of June and also at the end of August among different two-year-old unworked stocks. Moreover, there was some variation in the thickness of the strippings at the beginning of June and at the end of August. It was found that the bark could not be peeled before June or after August. Table XXVI shows the condition of the bark and the thickness of the strippings during each period.

TABLE XXVI.

Condition of Bark-peeling and Strippings on Different Stocks.

Stocks.	June 1st.		July 1st.		August 1st.		August 28th.	
	Condi- tion of bark.	Thick- ness of strip- pings.	Condi- tion of bark.	Thick- ness of strip- pings.	Condi- tion of bark.	Thick- ness of strip- pings.	Condi- tion of bark.	Thick- ness of strip- pings.
Quince A	Loose	Thick	Loose	Thick	Loose	Thick	Loose	Thick
Quince C	Loose	Thick	Loose	Thick	Loose	Thick	Loose	Thick
Quince F	Loose	Thinner	Loose	Thick	Loose	Thick	Loose	Thinner
Conference*	Loose	Thick	Loose	Thick	Loose	Thin	Loose	Thin
Durondeau	Loose	Thick	Loose	Very thick	Loose	Thick	Loose	Thick
Myrobolan B	Stiff	Thin	Loose	Thick	Loose	Thick	Stiff	Thick
Common								
Plum	Stiff	Thin	Loose	Thick	Loose	Thinner	Loose	Thin
Czar†	Stiff	Thin	Loose	Very thick	Loose	Thick	Loose	Thick
Victoria‡	Stiff	Thin	Loose	Very thick	Loose	Thinner	Stiff	Thin
St. Julien A	Loose	Thin	Loose	Thick	Loose	Thick	Loose	Thick
St. Julien K	Stiff	Thin	Loose	Thinner	Loose	Thinner	Stiff	Thin
Brompton	Loose	Thick	Loose	Thick	Loose	Thick	Loose	Thick
Hale's Early‡	Loose	Thick	Loose	Thick	Loose	Thick	Loose	Thick

* Grafted on Quince A.

† Grafted on Myrobolan B.

‡ Grafted on St. Julien A.

From Table XXVI it seems clear that in early summer and again in late August most of the scion varieties had thicker strippings than the stocks, and this would seem to indicate an earlier or quicker differentiation of the cambial cells. It is interesting to note that Conference Pear and Victoria Plum had thinner strippings than other Pear and Plum varieties, and in Victoria the bark was fairly stiff toward the end of August. This probably indicates an earlier cessation of cambial activity.

Among the unworked stocks studied, one interesting fact observed was that all the so-called incompatible stocks had either a later start or an earlier cessation of cambial activity, as indicated by the stiffness of the bark and the thickness of the strippings.

Bark condition and strippings were also studied on two-year-old compatible and incompatible combinations at the end of August. Both scion and stock just above and below the union were studied and the strippings compared. The results are shown in Table XXVII.

TABLE XXVII.

Condition of Bark-peeling and Strippings of Compatible and Incompatible Combinations.

Combinations.	Scion.		Stock.	
	Condition of bark.	Thickness of strippings.	Condition of bark.	Thickness of strippings.
Conference on Quince A ..	Loose	Thin	Loose	Thick
Conference on Quince F ..	Loose	Thin	Stiff	Thin
Durondeau on Quince A ..	Loose	Thick	Loose	Thick
Durondeau on Quince F ..	Loose	Thin	Very stiff	Thin
Czar on Myrobolan B ..	Loose	Thick	Loose	Thick
Czar on Common Plum ..	Loose	Thick	Loose	Very thin
Victoria on Myrobolan B ..	Loose	Thin	Loose	Thick
Victoria on Common Plum ..	Stiff	Thin	Loose	Thin
Hale's on Myrobolan B ..	Stiff	Thin	Very stiff	Very thin
Hale's on Brompton ..	Loose	Thick	Loose	Thick
Hale's on St. Julien A ..	Loose	Thick	Loose	Thick
Hale's on St. Julien K ..	Stiff	Thin	Very stiff	Very thin

It will be seen that the incompatible combinations, both scion and stock, had stiffer bark and thinner strippings than the compatible combinations. It is also interesting to note that in incompatible combinations, as in Durondeau on Quince F, the Quince F stock had much stiffer bark and thinner strippings than the scion Durondeau. The same phenomenon was observed on Czar on Common Plum, Hale's Peach on Myrobolan B and St. Julien K, and it seems to indicate that in the incompatible combinations the cambial activity of the stock is more or less independent of the cambial activity of the scion.

2. *Difference in period of callus growth between stock and scion.*

Callus growth has been studied in stock and scion parts after grafting. Both compatible and incompatible grafts of pears, peaches and cherries were made early in March 1936. In each case ordinary whip and tongue grafting was used.

Samples were taken weekly from each combination and examined under a dissecting microscope to observe the amount of callus growth both on stock and on scion. The regions and amount of callus growth in each sample were diagrammatically drawn to facilitate comparison, and are illustrated in Fig. 22.

It was observed that incompatible combinations showed greater variation in the amount of callus growth between stock and scion than the compatible ones. The amount of callus formed, however, is not always greater in the stock, as has been shown by Proebsting (44). The variation was particularly noticeable on very incompatible combinations, such as St. Julien K on Hale's Peach, *Prunus serrulata* on Mahaleb, and Quince F on Durondeau.

It was further noted that in incompatible combinations the position and area of the callus growth were somewhat irregular as between stock and scion. The callus cells appeared first in every case at the blunt or thick end of the slanting cut on the stock and scion; but in the incompatible combinations, the callus cells did not extend their growth to the thin end of the slant. On the other hand, in the compatible combinations, stock and scion extended their respective callus growths and soon covered the thin ends. In incompatible combinations the new callus cells of the stock were thus unable to get into contact with those of the scion, and in many cases, these cells died and turned black. Fig. 22 shows the exact positions and areas of callus growths as they were seen in fresh samples.

This variation in the periods of callus growth of the stock and scion seems coincident with the cambial activity described in the previous section. For instance, St. Julien K had a later stage of cambial activity than Hale's Early Peach, as shown in the strippings. It was observed in the callus study of the grafts that St. Julien K had much less callus growth, especially at the tip, in comparison with Hale's Peach when used either as stock or scion.

3. Variation in the periods of foliation and defoliation.

Studies have been made to see whether there is any correlation between the time of leaf unfolding and leaf fall in stock and scion and the degree of incompatibility.

To obtain the required data, stocks in the same plot and under the same treatment were chosen. The date of breaking into leaf was recorded for each stock: the number of leaves were counted, and the areas of a sample of ten leaves from each terminal shoot were measured in the first month after bud-break.

Although there were great differences in the times of bud-break, it was not possible to correlate them with incompatibility. For instance, Hale's Peach and Myrobolan B broke into leaf earlier than Brompton or St. Julien. There

was practically no difference in the date of leaf unfolding between Quince A and Quince F, both of which were much earlier than pear varieties. Similarly, the defoliation dates of the stocks showed no correlation with incompatibility.

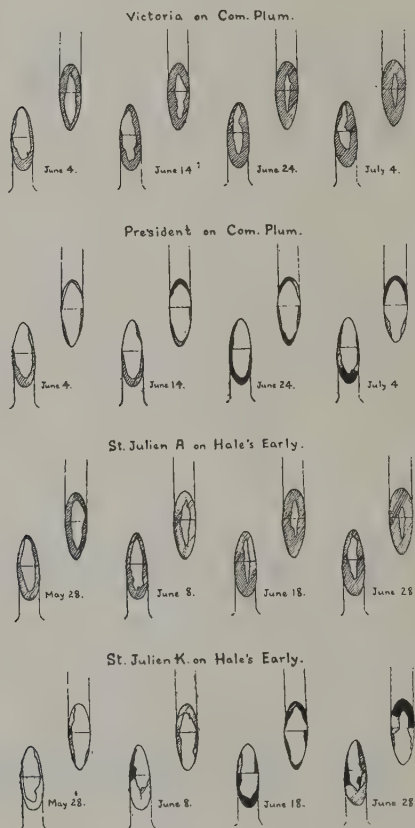


FIG. 22. Callus Growth of Compatible and Incompatible Grafts.

Shaded area—New callus.
Black area—Dead callus.

Thus, for example, Myrobolan B, a very late growing stock, has proved much more compatible with Czar Plum than has the early defoliating Common Plum stock.

When the date of bud-break and the rate of leaf-expansion were taken into consideration, it was noted that there was wide variation in the rate of leaf-expansion. For instance, Durondeau and Pitmaston pears expanded their leaves very much more slowly in the spring than Conference. Common Plum was about one month later in bud-break than was Myrobolan B, yet there was little difference between the two stocks in date of leaf-expansion. Similarly, Mahaleb F8/1/10, although showing bud-break about ten days later than Mazzard F5/1, expanded its leaves about ten days ahead of the Mazzard stock, as is shown in Table XXVIII.

TABLE XXVIII.

*Dates of Bud-break and Leaf-expansion of Pears, Plums, Peaches, and Cherries—
Two-year-old Stocks and two-year-old Scion Varieties.*

(Recorded in 1936.)

Varieties.	Date of bud-break.	Date of full leaf-expan- sion.	Rate of leaf expansion.
Durondeau on Quince A	March 20th	April 28th	Slow
Pitmaston on Quince A	March 15th	April 22nd	Slow
Conference on Quince A	March 10th	April 10th	Quick
Quince A (unworked)	February 20th	March 25th	Slow
Quince F (unworked)	February 15th	March 10th	Quick
Czar on Myrobolan B	March 10th	April 10th	Slow
President on Myrobolan B	March 10th	April 12th	Slow
Victoria on Myrobolan B	March 28th	April 20th	Quick
Myrobolan B (unworked)	February 20th	March 25th	Slow
Common Plum (unworked)	March 25th	April 5th	Quick
Hale's Early on St. Julien A	February 25th	April 7th	Very slow
Brompton (unworked)	April 1st	April 20th	Slow
St. Julien A (unworked)	April 1st	April 18th	Slow
St. Julien K (unworked)	March 28th	April 10th	Quick
Governor Wood on Mazzard	March 25th	May 4th	Very slow
Ukon on Mazzard	March 5th	April 20th	Very slow
Mazzard F5/1 (unworked)	March 20th	April 28th	Slow
Mahaleb F8/1/10 (unworked)	April 1st	April 18th	Quick

4. *Diploid and triploid pear grafts.*

Fortunately, the material available made it possible to ascertain whether any correlation existed between the chromosome make-up of stock and scion and incompatibility. In March 1936 Conference and Durondeau, two diploid pears ($2n=34$), were intergrafted and also reciprocally grafted with Pitmaston Duchess, a triploid variety ($2n=51$). The percentage take and the subsequent mean growth of the scions are given in Table XXIX.

TABLE XXIX.

Pear Grafting—Between Diploid and Triploid Varieties. All trees were worked on Quince A Stocks.

Combinations.	No. of grafts.	No. take.	% take.	First year shoot growth. cm.	S.E.
Pitmaston on Durondeau	30	20	67	131.5	15.9
Conference on Durondeau	30	30	100	118.8	7.5
Pitmaston on Conference	30	23	77	140.9	14.8
Durondeau on Conference	30	30	100	148.8	12.6
Durondeau on Pitmaston	30	29	97	111.2	15.9
Conference on Pitmaston	30	26	87	100.9	14.1
Durondeau on Durondeau	30	30	100	210.1	16.1

Although there are slight differences both in the percentage of take and in the subsequent average wood growth, yet they are not sufficiently significant to account for the degree of incompatibility.

Moreover, a chromosome study of Quince root tips has recently been made at the John Innes Horticultural Institution, showing that Quince A, C, F and G, which include two so-called compatible and two incompatible stocks, are all diploids, having a chromosome number $2n=34$. Therefore, the cause of incompatibility of Durondeau on Quince F, diploid on diploid, and the compatibility of Pitmaston Duchess on Quince A, triploid on diploid, evidently cannot be explained on the basis of the number of chromosomes.

5. *Annual growth curves of the unworked stocks and grafted scion varieties.*

The growth curves of the unworked and the grafted scion varieties were measured in the field. Stocks and one-year maiden trees (on compatible stocks) were planted in a uniform plot, having two replications in the row. Ten stocks of maiden trees were chosen at random for measurements during their second growing season. On each stock or tree, two terminal shoots were measured weekly in 1936 from bud-break until the cessation of growth. It was found that growth in length was fairly uniform within each set of ten trees.

The growth curves in Figs. 23-6 were prepared from these measurements. Each comparison of scion growth and stock growth on the graphs for grafted and ungrafted plants is the mean of measurements of twenty terminal shoots. Fig. 23 shows the weekly increments in shoot length for pears and Quince stocks and Figs. 24-6 show the total seasonal length for representative combinations in pears, plums and peaches.

It will be seen from Figs. 23-6 that the incompatible stocks had made not only much slower but also weaker growth. In addition, the growth curves of

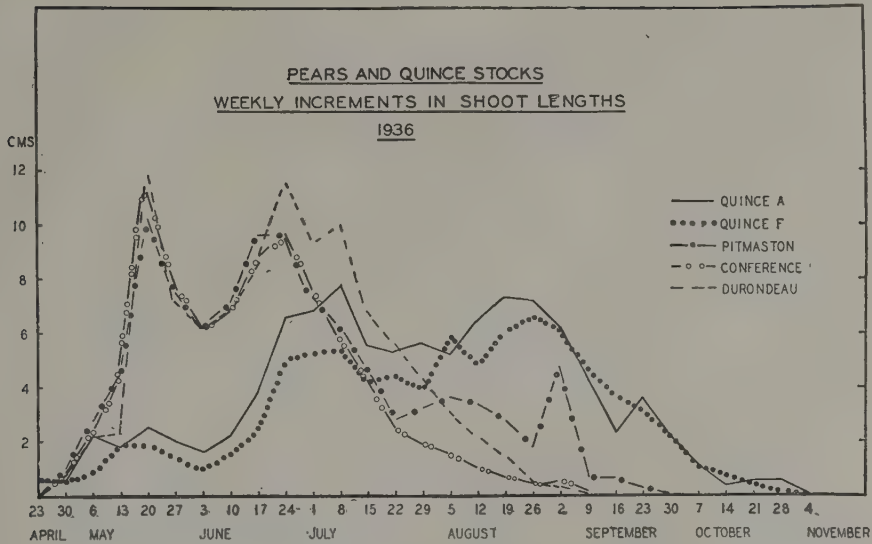


FIG. 23. Growth curves showing Weekly Increments in Shoot Lengths of Two Terminal Shoots. Pear and Quince Stocks.

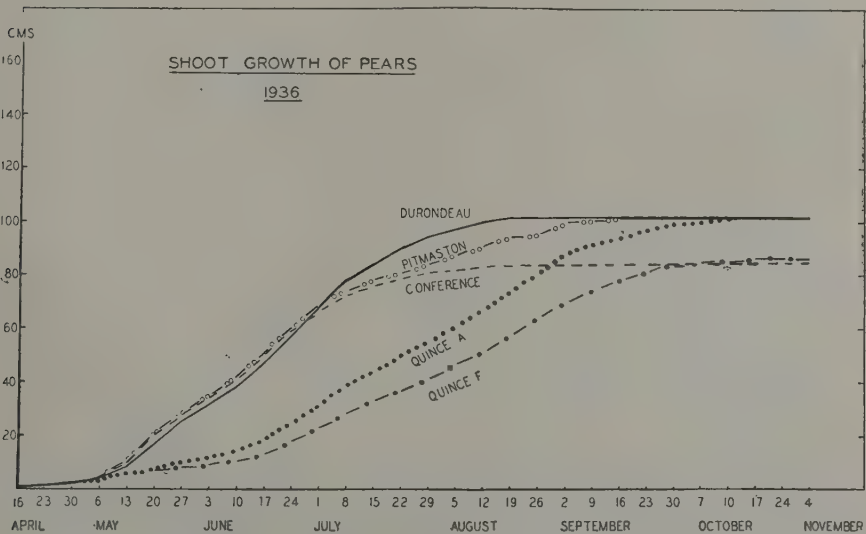


FIG. 24. Growth Curves showing Total Elongation of Two Terminal Shoots. Pear and Quince Stocks.

these incompatible stocks were quite different from those of the scions with which they proved incompatible.

In pears and quinces (Figs. 23-4) the curve for Quince F illustrates its slower and weaker growth as compared with that of Quince A and the three pear varieties. It is interesting to note that the growth of Conference, a variety which usually shows much delayed incompatibility, was complete very much earlier than that of Durondeau or Pitmaston Duchess.

Again, the growth curves of Victoria Plum and Common Plum stock (Fig. 25) are very similar, as also are those of Czar and Myrobolan B stock, two compatible combinations. But when the growth curves of Czar and Common Plum are compared very great divergence is apparent between the two, especially during the period May to July, when the trees were in their most actively growing condition. Although the growth curves of Victoria and Myrobolan B are also different, owing to the vigorous growth of Myrobolan B, they are much closer, as can be seen from Fig. 25.

The same phenomenon happened in peach and plum stocks (Fig. 26). Hale's Early Peach had a similar curve to that of St. Julien A, whereas it differed more from St. Julien K. The growth curves of Myrobolan B and Common Plum, two very incompatible stocks for Hale's Early Peach, differ strikingly from that of Hale's Early Peach.

When these growth curves were compared with the field observations, it was found that the incompatible combinations declined most rapidly during the period when the growth curves of the stock and the scion were at the most divergent stage. For instance, one-year-old Hale's Early Peach trees grafted on Myrobolan B, declined most rapidly at the end of July. The same phenomenon was observed on young grafts of Victoria Plum on Hale's Early Peach. At first, Victoria showed more vigorous growth than did Czar on peach stock, but about the middle of July, Victoria began to decline, and all the trees died at the beginning of September.

It appears (Figs. 23-6) that these differences in the rate and state of growth are of most importance during the main period of active growth. In all the incompatible combinations studied, the wide differences between their growth curves were most striking during May and up to the end of July. This is possibly due to the fact that during this period cell activity is at its height, and the wide differences in growth capacity at the union between stock and scion may then have a greater effect on the uniting tissues.

Finally, it was noted that when a weak growing variety was grafted on a strong growing stock, the grafts usually showed symptoms of incompatibility earlier and to a greater degree, than did those of a vigorous variety on a weak stock. This is shown clearly with Quince F on Durondeau, Common Plum on Czar, and Hale's Early Peach on Myrobolan B.

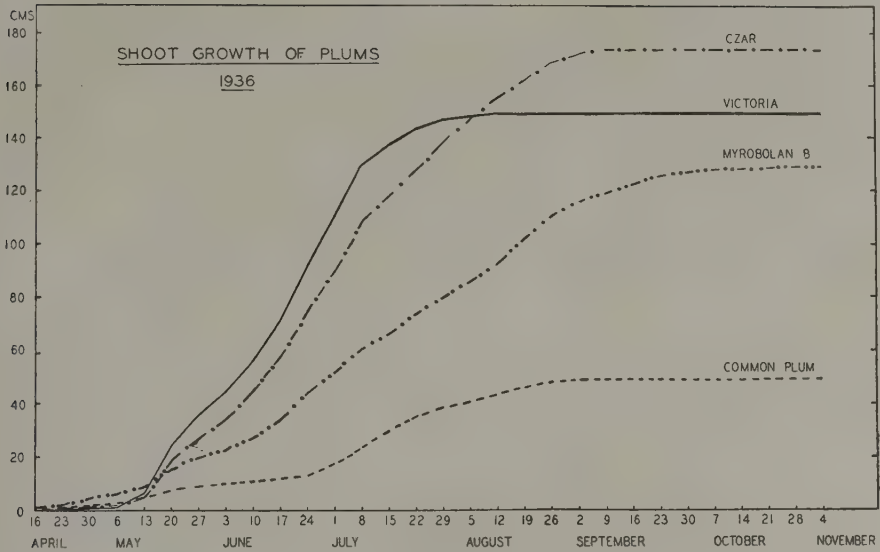


FIG. 25. Growth Curves showing Total Elongation of Two Terminal Shoots. Plum and Plum Stocks.

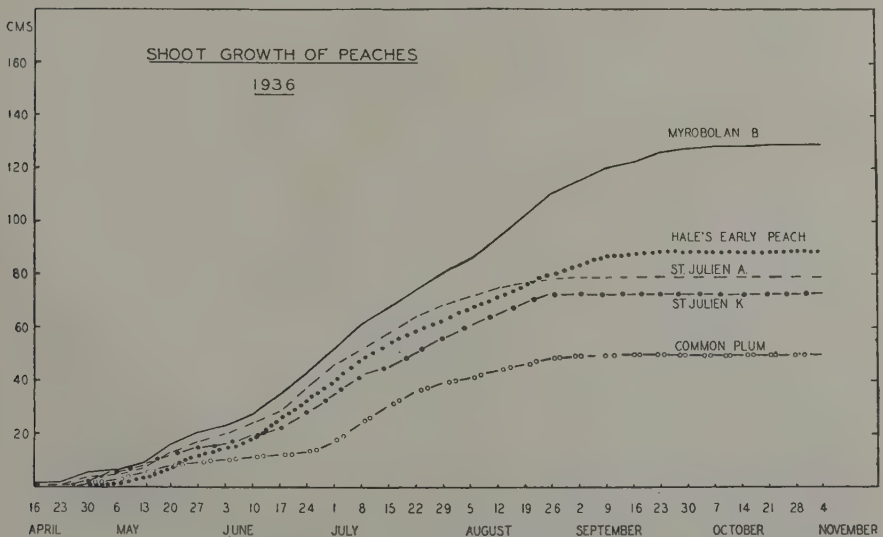


FIG. 26. Growth Curves showing Total Elongation of Two Terminal Shoots. Peach and Plum Stocks.

V. DISCUSSION OF RESULTS.

The nature of compatibility and of incompatibility.

In Citrus budding, Mendel (39) observed the following processes of union in a normal and compatible combination :

1.	First cell division	..	..	..	..	24 hours.
2.	First callus bridges	..	..	..	..	5 days.
3.	Cell differentiation (stock)	..	..	..	..	10 days.
	" " (scion)	..	..	..	..	15 days.
4.	First occurrence of tracheids (stock)	..				15 days.
	" " " (scion)	..				20 days.
5.	First occurrence of meristematic layers in the callus	..	..	..	..	15 days.
6.	Lignification of callus completed (stock)	..				25-30 days.
	" " " (scion)	..				30-45 days.

Thus, in order to form a well-established growing tree by grafting or budding, there must be a series of processes to ensure the junction of the functioning cells at the union. If the tree is to continue its normal development it should possess a healthy stem which can carry on the functions of an ordinary unworked tree trunk. Any conditions which hinder, obstruct or destroy these vital cell formations at the union are detrimental to the growing shoots and the roots, and will result in one or more symptoms of incompatibility.

All plants are capable of regenerating organs or tissues when they are cut off from the parent, but this power of regeneration is limited in extent. The uniting of stock with scion is accomplished partly by the regeneration of different sets of cells and partly by connecting together tissues performing similar functions belonging to the stock and the scion. Proebsting (43) observed that in the formation of new tissues the differentiation of the cells of the scion appears to be influenced by the cells of the stock adjacent to the scion cambium. That is to say, the cambium cells of the scion form medullary ray tissue when in contact with ray cells of the stock, and xylem tissue when in contact with xylem, etc. If these processes are all successfully completed the result will be a compatible union, resulting in a tree with a congenial and healthy stem. On the other hand, should there be any great difference in the capacity of regeneration between stock and scion, there would be a corresponding variation in the period of cell differentiation, since the new meristematic cells regenerated from the stock would probably find no similarly active cells with which to unite in the scion. The result of this would be accumulation of dead cells and abnormal tissue formation at the union. Such a combination of stock and scion is said to be incompatible.

The outstanding symptoms of incompatibility.

Growth decline.

Observations made in the course of the present study showed that incompatible combinations were sometimes associated with poor take of grafts and buds, especially in severe cases. They were always accompanied by subsequent decline in the area of foliage, in the amount of new wood, in new root growth and by other symptoms of ill-health, leading in most cases to the death of the tree.

Drying out of the stem and foliage, premature defoliation and premature flower bud formation seemed to indicate lack of water or of necessary nutrients in the scion.

In the experiments described, all the reciprocal (stock-scion and scion-stock) combinations revealed ultimately the same phenomena of incompatibility, but it must be noted that these results differ from those of Toxopeus (61) with *Citrus sinensis* and *C. Aurantium* grafts, and from those of Heppner and McCallum (24) with *Prunus salicina* and *P. domestica* grafts. These investigators did not get the same effects with reciprocal combinations.

Scion/stock ratio.

Vyvyan (63) observed that the total annual increment in weight of young trees was distributed in approximately constant proportions between roots, stems and leaves, irrespective of the size and exact age of the tree. Knight (31) showed that the ratio of stem increment to root increment is constant, within a given scion-stock combination, under East Malling conditions and as observed on winter pruned trees. He also showed that the scion/stock ratio is greater in larger than in small trees. The difference in the scion/stock ratio between compatible and incompatible combinations may therefore be due, at least in part, to the great difference in the size of the trees. When the scion proportion decreases, as shown in the decline of the annual wood growth in incompatible combinations, there would be a simultaneous decrease in the roots, and *vice versa*. But when the decrease on either side becomes too drastic, the other component cannot continue to function and death follows.

In the incompatible combinations, such as Durondeau Pear on Quince F or Hale's Early Peach on Myrobolan B, the scion portion was too small relative to the weight of the stock, as shown by the scion/stock ratio studies; and, as was found in excavations, many of the old roots died back without being replaced to any appreciable extent. As the scion growth continues to decline, more roots will probably die back, until finally a limit is reached when the root system can no longer function. Thus, as the tree grows older, decline both in shoot and root growth becomes more marked.

The initially high scion/stock ratio observed in incompatible combinations may possibly be explained by the higher food reserve in the scion, due to obstruction of the downward movement of food materials at the union, while the water supply from the roots is still sufficient.

Maskell and Mason (38) have proved that organic nitrogen accumulates in the bark and wood above the ring in ringed cotton plants, and Clements (8) has shown that mineral materials move upward in the xylem in girdled pears and plums. It seems that the discontinuity in the bark and wood of incompatible combinations, described earlier in the present paper, acted at first like a ring or girdle around the stem. Actually, starch deposits were found above the union in the incompatible Durondeau on Quince F, as illustrated in Fig. 21, therefore there was an initially higher scion/stock ratio. When the roots were deprived of sufficient organic nitrogen and other elaborated plant food, they started to die back, and so, in turn, the shoot growth of the scion was reduced. Hence, the scion/stock ratio of the incompatible combination was subsequently much lower than that of the compatible one.

The immediate causes of decline in incompatible combinations.

It has been proved in the present study that all the incompatible combinations had mechanically weak unions, as shown by bark and wood discontinuity, the presence of parenchymatous cell accumulations, the relative ease of breaking and the smooth nature of the fracture at the union. These abnormalities had actually caused obstruction of water, soil nutrients and food transport, as was shown by the dye injections, water conductivity tests, and the accumulation of starch above the union.

The correlation between mechanical weakness and abnormal cell accumulation at the union with obstruction to the flow of water was suggestive. The experiment with negative pressure confirms Knight's (28) observation with positive pressure tests and proves that there was a big increase in resistance to the passage of water resulting from defective unions.

Abnormal cell enlargement and cell accumulations in incompatible unions were found on microscopical examination to be present within a few weeks after budding.

It is known that for grafts to be successful the scion must be kept as moist as possible to prevent drying out before actual union takes place. Therefore, the primary need of the scion is a sufficient water supply. In the early stage of union, however, the water requirement of the scion is small and sufficient water may be transported through the enlarged and irregularly arranged callus cells. But when the tree grows older and the scion becomes larger, the water transported through an obstructed union, together with a much restricted root development,

is insufficient to meet the increasing demands of the scion, especially during hot summer periods. Thus, the scion becomes dry, and finally dies back as a result of desiccation. This is most obvious in Common Plum on Czar, and Hale's Early Peach on Myrobolan B. The autumn defoliation of all incompatible combinations started from the tops of the branches instead of from their bases. The root system had apparently declined so much that there were not enough new roots to ensure a sufficient supply of water to the tops.

In peaches, excessive gum formation is probably an after-effect of the sudden change in the moisture content of the stem. This excessive gum formation inside of the conducting vessels will of course hasten the death of the scion. It seems to explain why Hale's Early Peach on Myrobolan B and Myrobolan B on Hale's Early Peach died back more rapidly than incompatible combinations of pears and plums, where gum formation was absent.

The possible fundamental causes of incompatibility.

Previous workers (15, 46, 14, 16) have shown that between plants of distantly related groups there are significant differences in nutritional capacities and in enzymatic or precipitin reaction. This bio-chemical aspect of the problem has not been included in the present investigation which is concerned only with plants of related groups.

On the physiological side, it has always been difficult to explain why the same variety should be compatible with one selection of stock and incompatible with another. Field observations showed indications of the existence of wide differences in certain physiological functional capacities between incompatible and compatible stocks and scions, as has already been discussed. The author therefore devoted more attention to some of these physiological functional differences in the deciduous fruit trees investigated. Incidentally, some interesting results have been obtained as described in the previous sections.

Period of cambial activity.

The present investigations showed that there were differences in the periods of cambial activity between the scion and its corresponding incompatible stock, as shown by the peeling of the bark and by strippings, using the method described by Priestley (42).

Knight (29, 30) and Swarbrick (59) observed on apple and plum trees, that cambial activity starts in the region of buds that are developing into new shoots, and spreads downwards from these through the stem. Moreover, Haley (18) found that there was more rapid or slower spread of cambial activity and vascular differentiation in the stems of different forest trees such as *Quercus* and *Aesculus*.

With these results in mind, it is not surprising to find that when a scion variety of late or slow cambial activity is grafted on a stock variety of early or rapid cambial activity, constant breaking and "re-grafting" of the uniting cells takes place and that a large accumulation of inert functionless tissue occurs at the union, such as has been found in incompatible combinations.

However, contrary to the statement made by Swarbrick (59), to the effect that the cessation of cambial activity works basipetally in the stem of an ordinary healthy tree, the results of the present experiments would appear to show that in incompatible combinations, such as Czar on Common Plum, or Durondeau Pear on Quince F, the cessation of cambial activity in the stock starts from the union and proceeds downwards, somewhat independently of the cambial activity in the scion. In these cases (Table XXVII), the stocks showed a stiffer bark and thinner strippings much earlier than did the stem portion of the scion just above the union.

The duration of leaf-expansion after bud-break, as observed in the present investigation, may be associated with cambial activity. The shorter period between bud-break and leaf-expansion observed in Common Plum and Mahaleb seems to indicate more rapid cambial activity in the tree (Table XXVIII).

Period of callus growth.

The callus studies of grafts, already described, also revealed functional differences in the efficiency of callusing between different stocks and scion varieties. Sharples and Gunnery (56) stated that the cambium plays no part in the early development of callus, which is predominantly the product of medullary ray cells. It seems certain, however, that all the water supply of the scion has to be transmitted from the stock through this "transitory callus bridging". Callusing is in fact the necessary first step for the formation of permanent conducting tracheids, as Mendel (39) observed. If either the scion or the stock were delayed in its period of callus bridging, there would also be a delayed period of tracheid strand differentiation. In the absence of corresponding connecting tissues, the earlier growing callus and tracheid strands would dry out and become dead cells, as shown in Fig. 22. When the callus tissues from the delayed scion or stock begin to grow, they find no corresponding meristematic cells with which to unite. Sorauer (58) pointed out that in setting grafts, actual contact of cambium layers is not essential, and that it is in practice rarely achieved, but contact of cambium-derived callus is important. Thus, if the variation in time in the regeneration of callus tissue between stock and scion is very great, the scion will not grow or will make a very poor start and then dry out. But if the variation is less pronounced there will be constant breaking and re-grafting of the tissues which are uniting with each other. Thus, a thick layer

of enlarged cells or cell accumulations will be formed, or there will be inwardly curved layers of cells as manifested in the clean breaking-out of incompatible combinations (Fig. 18). Both scion and stock will be weakened by this obstruction at the union.

Growth curves.

When the growth curves, representing the extension growth of two terminal shoots, were compared it was observed that there were differences not only in the times of starting and cessation of growth but also in the periods of most rapid growth. As already discussed, the combinations were generally incompatible when the growth curves of the stock and scion were most unlike. The best example can be seen in Czar on Common Plum. It may further be proved in Victoria and Czar plums grafted on Hale's Early Peach. Czar showed a similar growth curve, and Victoria a very different one, compared with Hale's Early Peach. Eventually, all Victoria on Hale's Early Peach were defoliating and dead by the end of the first season. The decline in growth in the incompatible combinations was usually most rapid during the period when the growth curves showed most divergence.

Moreover, incompatibility appeared earliest and in its most acute form in those combinations in which the growth curve of the scion was weaker than that of the stock. This may be due to the normal variation in growth activity between a scion and a well-established rootstock. In the reciprocal combinations in which the growth curve of the stock was the weaker, the symptoms of incompatibility are delayed.

The fact that the degree of incompatibility was more severe in older trees may be explained by the breaking down of the tissues at the union in successive seasons. As the tree grows older and taller, these growth activities starting at the growing tips have to travel a much longer stem before they can reach the union. Thus, the variation in the time of commencing and cessation of these growth activities between the cells of the scion and stock at the union will be greater. Swarbrick (59) noticed that in apple branches, the initiation of xylem formation began in the apical regions and worked downwards, there being at least three weeks' difference between its initiation in the one- and the four-year-old branches.

It is reasonable to assume, therefore, that these differences in cambial activity, in capacity for callus tissue generation, in growth curves and in the rate of transmission of these activities through the stem form an important part of the causes of incompatibility.

Indeed, it is a common practice to store scion wood before grafting in order to get delayed dormancy and thus to synchronize the cambial activity and callus

growth in scion and stock. This result is also sometimes attained by treating the scion or the stock with certain chemicals so as to stimulate the growth activities of the cells at the union. Gossard (13) found that he could get a higher percentage of graft take by treating the scion wood with chemicals. But since all these physiological functional capacities are inherent, the successful uniting together of the conducting cells by artificial inhibition or stimulation is only temporary and will break down again in the following season.

All these physiological growth activities are extremely susceptible to the influences of soil and climatic conditions, as is observable in the field. Therefore, it is not surprising to find contrasting cases of compatibility and incompatibility with the same given scion and stock in combination. Work done at East Malling (1) has shown the variable results obtained in the nursery from year to year. Swingle (60) and Shippy (57) showed that the callusing of apple varieties differed in regard to optimum temperature requirement, as well as in the duration of maximum activity and the dates of beginning and end. Again, Howard and Heppner (27) showed that some strains of Myrobolan Plum were generally successful as peach stocks in California. But when these Californian strains of Myrobolan were sent to East Malling and were worked with Hale's Early Peach scions, none of them showed any sign of compatibility with this variety.

Moreover, since all these growth activities are inherent, they may be transmitted and segregated in the progenies after crossing two parent varieties. Crane has informed the present author that he observed on crossing Durondeau with Dr. Jules Guyot (a pear variety which usually fails on nearly all quince varieties), that there were a few seedlings in the F_1 generation which were incompatible with Quince C.

From the practical point of view, slight incompatibility may render a tree less vegetative in its growth and more prolific in bearing. As with pears on Quince A and Quince C, there was noticeable discontinuity in the outer cortical region whilst both the bark and wood fibres were continuous (see Fig. 13). Such slight discontinuity may have a similar effect to ringing or girdling, as sometimes practised on extremely vigorously growing trees. Kraybill (36) has proved that ringing lowers the moisture and total nitrogen content above the ring, but increases the reducing sugars, thus resulting in a higher carbohydrate/nitrogen ratio. Again, du Sablon (54) has shown that there is a higher starch content in Duchesse d'Angoulême pear scion in all seasons, when grafted on quince than on pear stocks.

Thus, it would even appear that the use of a rootstock which is not entirely compatible may sometimes be preferable, from the growers' point of view, to that of a stock which shows no incompatibility. But it would be highly detrimental to grow trees on very incompatible stocks, a proceeding which may result in quick decline and premature death in the nursery and orchard.

SUMMARY.

1. The problem of incompatibility between stock and scion has been studied in deciduous fruit trees, especially in pears, plums, peaches and cherries. Some 86 grafted and 32 budded combinations of scion-stock, stock-scion and stock-stock have been studied at East Malling during 1935-37. All the root-stocks used were of clonal origin and were vegetatively propagated.

2. With all incompatible combinations, grafted or budded, there were marked symptoms of a low percentage take, premature autumn leaf-coloration and flower bud formation, early defoliation, and dying back of young shoots. The low percentage take was less noticeable on budded than on grafted plants. Defoliation started from the tops of the shoots in incompatible combinations in contrast to the ordinary defoliation from the basal portion of the shoots in compatible combinations.

3. In all incompatible combinations, there was a marked gradual decline of the annual wood growth, usually following an initially vigorous growth at an earlier stage. There was also a gradual decline of new root growth both in number and length. The incompatible combinations started new root growth much later in the spring than the compatible ones. These findings corresponded with the results obtained from excavated trees, namely that the incompatible combinations had an initially higher but subsequently much lower scion/stock ratio than the compatible ones. All these symptoms were intensified in direct relation to the age of the tree and the degree of incompatibility. Moreover, these symptoms occurred both in scion-stock and their reciprocal combinations.

4. All incompatible combinations showed a mechanically weaker union than the compatible ones. This was indicated by :

(a) Bark and wood-fibre discontinuity. Incompatible combinations showed three classes of bark and wood-fibre discontinuity, arranged in order of decreasing severity, as follows :

- (i) Both bark and wood fibres discontinuous.
- (ii) Bark continuous, but wood fibres discontinuous.
- (iii) Bark discontinuous, but wood fibres continuous.

(b) Heavy accumulation of parenchymatous cells at the union. Compatible combinations possessed regular strands of medullary ray cells across the union about four weeks after budding ; whereas the incompatible ones had much enlarged parenchymatous cells arranged irregularly at the union even up to twelve weeks after budding.

(c) Reduced strength at the union and clean surface of break. Incompatible combinations required far less force to fracture the union than compatible ones,

and the surface of the fracture was invariably smooth and had a distinctive layer of inwardly curved cells.

5. Incompatible combinations had some form of obstruction at the union. This was indicated by :

(a) A slower flow of dyes through the union, as shown by dye injections.

(b) A much higher resistance to the flow of water, as shown by water conductivity tests.

(c) A heavy deposit of starch above the union in November, as shown by the iodine test.

Mechanical weakness and obstruction at the union are suggested as the immediate causes of the decline of a tree showing an incompatible combination.

6. Some of the physiological functional differences between the scion and its incompatible stock have been studied. It was found that different stock and scion varieties had :

(a) Different periods of cambial activity, as observed by the bark-peeling and stripping method, in summer and autumn.

(b) Different periods of callus growth and different regions of callus differentiation, as studied in grafts. In incompatible combinations, most of the callus cells which were not in contact died after about a month.

(c) Difference in the duration of leaf-expansion from the time of bud-break. The combination was most incompatible when a slow leaf-expanding scion was worked on a quick leaf-expanding stock.

(d) Difference in growth curves, as shown by the weekly measurements of the terminal shoots.

The wider the divergencies of such physiological functional differences between a scion and its stock, the greater the degree of incompatibility.

7. It is concluded that a mechanically weak union and the increasing obstruction at the union are the immediate causes of shoot and root decline in an incompatible combination, and that all the physiological functional differences manifested in cambial activity, callus growth, duration of leaf-expansion, and growth curves of the stock and scion varieties may be accounted as important contributory causes of incompatibility among deciduous fruit trees.

8. It is pointed out that all these physiological functional differences in the growth activities of the stock and scion are inherent, and may be somewhat modified under different climatic and environmental conditions, and this helps to explain the different results obtained in different parts of the world in regard to the occurrence of the symptoms of incompatibility.

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A STUDY OF THE TECHNIQUE OF VARIETY TRIALS, AS ILLUSTRATED BY THE COMPARATIVE YIELDS OF FOUR BLACK CURRANT VARIETIES GROWN IN THREE DIFFERENT LOCALITIES

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THE adequate testing of new varieties of commonly grown fruits is a difficulty with which modern horticultural research has to contend, and in recent years it has become more and more exacting. The flow of new varieties is unceasing ; and for the protection of the grower it is essential that their qualities should be carefully tested by an impartial authority. While the final decision as to whether or no he adopts a new introduction must rest with the grower himself, much can be done to assist him by passing the flow through a series of sieves in order to eliminate all but a few of the most worthy.

Such a grading system has been provided by the National Fruit Trials which, for the past sixteen years, has been employed by the Royal Horticultural Society under the aegis of the Ministry of Agriculture.

The varieties are sent in to the Society's gardens at Wisley and are there kept under close observation for a period of years in comparison with standard varieties of proved quality and yielding capacity. The plan of this preliminary testing need be only of a simple nature, for the differences to be looked for are chiefly in the quality of the fruits and on a large scale ; and unless a new introduction shows some marked improvement on existing sorts, it need be considered no further. It is necessary only to have sufficient plants for each variety to be fairly represented, and to arrange them in such a way that the sources of error to which they are subject are comparable.

From this first test a few varieties may emerge good enough to undergo a much more severe trial. Are they in fact worthy of introduction in commerce ?

While certain varieties may be excellent for garden use, the demands of the market grower are often very different. Mere excellence of flavour is for him not sufficient—indeed there are certain attributes which to him are equally, if not more important, and the chief of these is yield. Further, while a variety may give good returns under the sheltered conditions of a private garden, how will it survive an altogether harder existence in the open field ? And will it "do" in his field ? In other words, is the variety of general value or useful only under particular conditions of soil and climate ?

These questions are very much more difficult to answer, involving, as they do automatically, a series of trials in as many places as possible. Further, the performance of varieties in commercial use to-day is of a high order, and increases in yield are likely to be comparatively small. Not that these increments may not be of commercial value, but a much more rigorous test is necessary to establish or disprove their existence.

It was in order to find a suitable method for this kind of trial that the present series of experiments was initiated in 1927 by the Joint Committee who administer the scheme, in collaboration with the research institutions concerned.

MATERIAL.

For the time being the investigation was confined to small fruits, and the black currant was adopted as a suitable indicator crop.

Only three stations collaborated in the work, since, in order to find the best method, the trials planned were much more elaborate than was then deemed necessary, and these stations alone had the facilities for recording and the land available. The three research institutions concerned were the Royal Horticultural Society at Wisley, Surrey, the Long Ashton Research Station, Bristol, and the East Malling Research Station, Kent.

The soil at Wisley is a sandy loam with a similar but slightly heavier subsoil, and the station has an annual rainfall of about 25 inches. Long Ashton is situated on a fine sandy loam overlying a marly subsoil, and has an annual rainfall of 34 inches; while at East Malling the soil is a good medium loam over the Kentish ragstone, and the rainfall averages about 25 inches.

The varieties chosen were two standards, well known in commerce, Boskoop Giant and Baldwin, and two new introductions which had already passed a preliminary test at Wisley, Davison's Eight and Taylor's 21/23. The two standards were selected to represent early and late bearing varieties. While they are typical of the varietal groups to which they belong, they do not cover the whole range of black currant varieties, representatives of the "French" and "Goliath" groups being absent. There was thus no strict comparison, for instance, between Davison's Eight, which is nearest to the Goliath group though of the same season as Boskoop, and a standard variety of the same type. One hundred bushes of each variety were distributed to each of the three participating stations, all the material having been propagated at Wisley.

THE EXPERIMENTAL DESIGN.

In 1927 information as to best size of plot and number of replications was not very extensive. Hatton and his co-workers had recently published their paper (1) on Black Currant Variety Trials and had stated that fifty-seven bushes of each variety would be required to prove a mean difference of 10% to be

statistically significant, these figures being based on the totals of three cropping seasons.

However, the bushes in that trial were not so arranged that the effect of soil differences could be eliminated ; and there was no information as to the accuracy which might be achieved by the use of the then fairly new methods of plot arrangement, such as the Latin square, recently developed by Fisher at Rothamsted. Accordingly, in the present experiment, it was decided to allow ample numbers, so as to hit the optimum as nearly as possible and to try how far a randomized pattern could be adapted to the needs of the trial.

The next point to be decided was the experimental unit. In experiments with trees in England it is usual to adopt the individual tree as the equivalent of an agricultural plot. However, to distribute one hundred individual bushes of each of four varieties at random would have been an obvious absurdity, and a plot of four bushes was adopted as the smallest unit which could conveniently be harvested and recorded.

The four varieties were then arranged in a series of six four by four Latin squares, each square containing four plots of four bushes of each variety. There were thus ninety-six bushes to be recorded in twenty-four plots of four bushes for each of the four kinds. It is hardly necessary to explain the nature of the Latin square design now, but it may be stated briefly that in it there are the same number of plots of each variety as there are varieties, and that each variety occurs once in each row and once in each column of a square pattern. It is also part of the definition that each new square used should be picked at random from all possible squares which fulfil the first two conditions.

In the present experiment the six such squares were to be laid down adjacent to one another at each station. This was carried out at Wisley and at East Malling, the only difference between the two being that at the latter station the individual rows of black currants were interplanted with raspberries. This may have increased the plot to plot variability, owing to varying competition with the intercrop. At Long Ashton it was not possible to carry out the scheme exactly ; owing to the shape of the ground only four squares were possible, and two sets of four randomized blocks. Further, the four squares used were identical in pattern.

The three trials were planted in the winter of 1927-28, and annual records of yield were taken until the summer of 1933. Omitting the few fruits in 1928, the following discussion is based therefore on the yields of four-bush plots in five consecutive seasons.

THE RESULTS.

(i) *Individual Trials.* The results were examined by the analysis of variance method (2), and the following Table shows the average yield over the

whole period in kilograms per plot of the four varieties at each station, together with the standard error appropriate to the averages in each trial, and the difference necessary to give a 20 to 1 chance in favour of significance. In order to make a comparison of the three trials, these varietal averages are also given as percentages of the mean yield of all four varieties in each.

TABLE I.

Total crop for five-year period in kilograms per plot of four bushes and as a percentage of the general mean of all four varieties at each station.

		Boskoop.	Baldwin.	Davison's.	Taylor's.	Gen. Mean.	S.E.	Sig. Diff.
Wisley	Kilos	26·7	21·8	15·6	25·5	22·4	0·48	1·4
	%	119	97	70	114	100	2·1	6·2
Malling	Kilos	21·5	31·3	18·3	2·0	18·3	0·48	1·4
	%	117	171	100	11	100	2·6	7·6
Long Ashton	Kilos	34·7	24·8	14·3	12·2	21·5	0·61	1·7
	%	161	115	67	57	100	2·8	8·1

The chief point of interest in this Table is the accuracy of the results. The last two columns show the standard errors and the differences between means necessary for significance. These last vary from about 6% at Wisley to 8% at Long Ashton. As might be expected from the large number of replications and accurate technique, the differences which the experiment is capable of establishing are fairly small compared with those obtained in many horticultural trials. That at Wisley gives the best result, probably because only here was the full benefit of the Latin square design felt. At East Malling, probably owing to variable interplanted raspberries, the variations between like plots were not markedly connected with definite positions in the field. Thus the effect of position could not be eliminated in the statistical analysis to any great extent. At Long Ashton, again, a somewhat less accurate layout was used, so that both these stations gave a rather lower degree of precision than Wisley.

Still, regarding these as individual trials, it is possible to say that at each one of these stations the method employed was capable of giving a comparison of the four varieties, over the period concerned, of very fair precision. In actual fact very large differences in yield were observed at all three stations, but even if they had been of a much smaller order, a definite ruling could have been obtained on any difference of commercial importance.

But there is much more to be learnt from these results than this. Up to this point they have been considered as the results of three unconnected

trials. If this had really been the case, it would not have been possible to argue further, for any difference in the response of the varieties to particular conditions at these three stations might have been attributed to the material, the age of the bushes or a difference in planning. But in the present example these three factors are practically identical, except for small deviations in the last, which have already been shown to have decreased only slightly the accuracy at the stations concerned. It is therefore perfectly justifiable to analyse the three trials as a whole. This will yield very much more information; for now it is possible to evaluate accurately the response of the four varieties to varying conditions of soil and climate. Table II (a) tabulates the mean yields for the five-year period, this time as a percentage of the mean yield of all stations and all varieties.

TABLE II (a).

Average yield of four varieties for all stations, as percentage of the mean of all four.

Boskoop.	Baldwin.	Davison's.	Taylor's.	Gen. Mean.	S.E.	Sig. Diff.
134	125	77	64	100	1.5	4.2

In this Table the yields are those of the seventy-two plots of each variety at the three stations. It may be asked, what is the value of an average yield from these so widely different places? If a new variety is to be of commercial value it must succeed in a majority of those districts in which black currants are likely to be grown. If such is the case, it should yield a higher average, taking into account all the places tried, than others in the trial. The above Table gives an accurate comparison of the merits of the four varieties for general purposes, in so far as the yields of three stations can give that information. Evidently the greater the number of stations, the greater the value of these grouped yields.

Naturally, since there are three times as many plots, the accuracy is greater than that obtained at a single station, the difference necessary for a 20 to 1 chance being reduced to 4.2. It is evident that on the whole the two varieties have not stood the test of comparison with the two established varieties Boskoop and Baldwin, and cannot therefore be recommended for general use. Of the two former, Boskoop has been the heavier cropper, while Davison's has yielded more than Taylor's. But, again, this is by no means the whole story, as is shown in Table II (b).

Although unsuitable for general introduction the two new varieties may have a value for particular soils or climates. The analysis in this case showed clearly that although the grouped yields showed definite differences, the relative yields of the four varieties were not alike for all stations.

In this Table the relative mean yield of each is that of twenty-four plots, as in Table I, and the significant difference, as might be expected, is intermediate between those of the three stations.

TABLE II (b).

Average yield of the four varieties at each station.

	Boskoop.	Baldwin.	Davison's.	Taylor's.	Gen. Mean.	S.E.	Sig. Diff.
Wisley	129	105	75	123	100	2.6	7.3
East Malling	104	151	88	10			
Long Ashton	167	120	69	59			

It is quite clear that the varieties have responded differently to the conditions in these localities. Boskoop and Baldwin have both been good in all three places, though not always in the same order. The former has cropped the better at Wisley and Long Ashton, while the latter was considerably the heavier cropper at East Malling. Of Davison's it can be said only that its performance has been generally mediocre, although it may still be an improvement on other varieties of the same varietal group. But in comparison with Boskoop and Baldwin, unless it has some outstanding quality other than yield, it is hardly worth introduction. Taylor's, on the other hand, appears to be much more particular, being outstanding at Wisley, poor at Long Ashton, and a complete failure at East Malling.

The reasons for these varietal preferences are more difficult to define, and, as they are not material to the present study, will not be considered here. However, a consideration of the annual crops, depicted graphically in Fig. 1, shows that these results are fairly consistent from year to year, the relative cropping of the varieties being roughly the same both in good and bad seasons. The only marked departure is the comparative behaviour of Boskoop and Baldwin in different years at East Malling, and the marked improvement of Davison's as compared with Taylor's at Long Ashton in the last two seasons.

It may be considered therefore that, in so far as the five seasons during which the experiment was in progress are concerned, the relative cropping value of these varieties for the three stations under the conditions under which they were grown, is definitely established.

How far are these results of general applicability, and if they are not, does the experiment give any guidance for such trials in the future?

Evidently this three-fold trial has given results of much wider application than merely a single trial could have done, but it is obvious that, as far as the fruit growing areas of England are concerned, a wider range of soils and

climates would add very greatly to the confidence with which the findings could be regarded.

To carry out such trials as these, however, would be beyond the powers of any but fully equipped and staffed experimental stations. Nor are such accurate

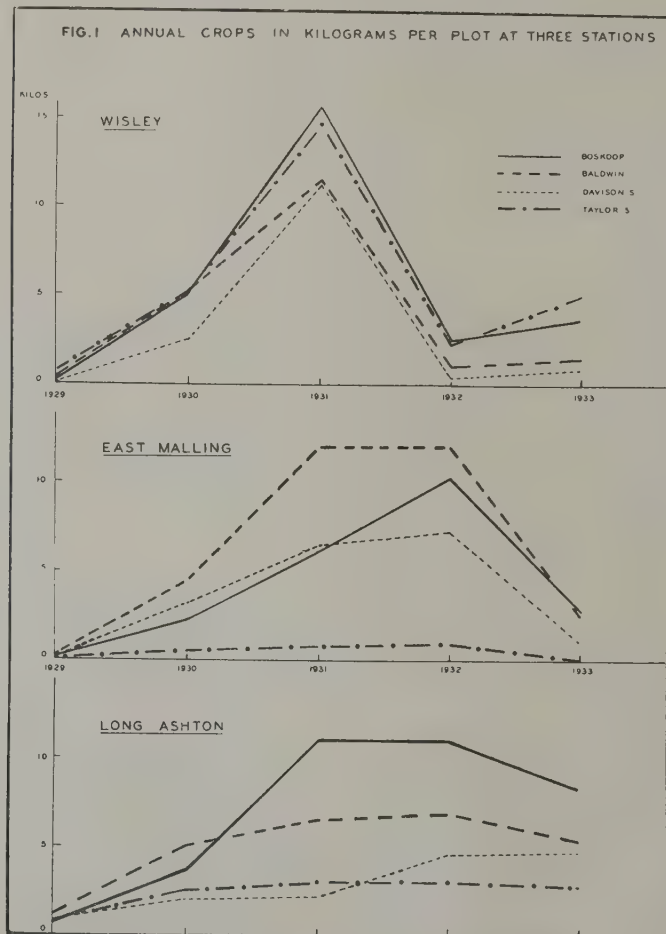


FIG. 1.

trials necessary. A series sufficiently precise to prove definitely the occurrence of increases in crop of 10% over a wide range of stations or 15% at a single station would probably be ample for present purposes; since, if there is no

greater difference than this, qualities other than yield are likely to be the deciding factor as to a variety's adoption or rejection.

The present data will therefore next be used to indicate the accuracy which might be attained with varying sizes of experiment at a single station, and the improvement to be obtained by increasing the number of stations.

For the present a single four by four Latin square is taken as the smallest arrangement for comparing four varieties, and the effect of increasing this from one to six squares is shown. It may be noted here that the four-bush plot is not necessarily the most economic unit and, where land is a smaller consideration than labour, it might be better to increase the plot size rather than the number of replications. However, the present experiment does not readily lend itself to a discussion of size of plot, and only the four-bush plot will be considered here.

EFFECT OF INCREASING REPLICATION ON PRECISION.

The precision of an experiment depends upon three factors, the variability of the material, the number of replicated plots, and the method of arrangement. In this case the last factor is constant, i.e. the four by four Latin square, so that for present purposes it is necessary to consider only the first two.

In planning an experiment two points must be considered. There must first be sufficient repetitions of like plots to give a reasonably good estimate of the variability of the material, and secondly there must also be enough to give a reliable estimate of the mean yields of the varieties in the trial. If the first condition can be predicted in advance, it is possible to estimate very closely how many repetitions are necessary to give any desired degree of accuracy in the estimates of mean yield.

The value usually employed to give an estimate of the variability of the material is the coefficient of variability (V). This is simply the standard deviation (σ) as a percentage of the general mean of all plots. Having obtained an estimate of the standard deviation, the errors to which any particular mean is subject are calculated by dividing by the square root of the number of repetitions of which it is composed. This is known as the standard error of the mean. In considering the difference between any pair of means in the experiment the standard error of the difference must be found. This is the standard deviation divided by the square root of half the number of repetitions, where this number is the same for each variety. In order to estimate the significance of any particular difference, it is divided by its standard error to obtain a figure known as t . Tables are available (2) in which can be found the value of t necessary to give a 20 to 1 chance in favour of significance. t varies according to the size of the experiment. Where it is on a large scale the value is about two, but if the experiment is more limited t must be rather higher to give the same level of significance.

Thus, if V is known it is a simple matter to calculate not only the expected standard error of the mean yields, but also the percentage difference, the significance of which any particular experimental design may be expected to prove, i.e.

$$\text{Standard Error (per cent.)} = \frac{V}{\sqrt{n}}$$

$$\text{Significant Difference (per cent.) } (P=0.05) = \frac{V}{\sqrt{\frac{1}{2}n}} \times t.$$

Where n is the number of replications.

(Note, a probability ($P=0.05$), gives odds of 20 to 1 in favour of significance.)

In the following Table III the percentage standard errors appropriate to varietal means, which would be expected at two individual stations, are tabulated for designs embodying from one to six four by four squares. The best estimate of the coefficient of variability at each station is that obtained when the yields of all six squares are analysed together, and this is used for the calculation.

TABLE III.

Comparison of the precision expected and obtained by increasing numbers of four by four Latin squares at two individual stations.

	Number of Squares.					
	1	2	3	4	5	6
WISLEY						
(No. of Plots)	(4)	(8)	(12)	(16)	(20)	(24)
Standard Error %						
Expected ($V=10.6$)	5.3	3.7	3.1	2.7	2.4	2.1
Obtained	5.7	3.1	3.3	2.7	2.4	2.1
Significant Difference % ($P=0.05$)	19.5	9.4	9.5	7.9	7.0	6.2
EAST MALLING						
(No. of Plots)	(4)	(8)	(12)	(16)	(20)	(24)
Standard Error %						
Expected ($V=13.0$)	6.5	4.6	3.8	3.2	2.9	2.6
Obtained	6.2	4.7	3.9	3.6	3.1	2.6
Significant Difference % ($P=0.05$)	21.7	14.3	11.3	10.3	8.9	7.7

With these are compared the actual standard errors obtained by taking at random, for analysis, first one square, then two squares, and so on until all six have been used from each station. From these latter are calculated the actual significant differences which each of the six combinations was able to prove. Thus, in a single experiment at one station, it might be expected to prove differences between means of about 20% if a single four by four square were used. If the size of the experiment were doubled, this would be decreased to about 13%, and if trebled, to about 10%. Subsequent additions do not greatly

increase the accuracy, the significant difference decreasing about 1% for each new square added. It is interesting to note that on the whole the standard errors obtained agree very closely with those expected. The larger deviations occur where the number of squares is small. This may well be expected, for with so few replications the estimate of the coefficient of variability would not be very reliable.

It is of interest to compare these results with Hatton and his co-workers' fifty-seven bushes to show a 10% difference, mentioned earlier. This estimate apparently agrees fairly closely with the new results at East Malling. (Between three and four squares, i.e. about fifty-six bushes.) Where, however, the Latin square design was able to work more efficiently at Wisley, three squares, i.e. forty-eight bushes per variety, would have been ample to give the same precision.

TABLE IV.

Comparison of precision expected and obtained by increasing numbers of squares when two stations are analysed together.

Number of Squares Number of Replications	1 (4)	2 (8)	3 (12)	4 (16)	5 (20)	6 (24)
AT EACH STATION						
Standard Error %						
Expected ($V=11.7$)	5.8	4.1	3.4	2.9	2.6	2.4
Obtained	5.9	3.8	3.5	3.1	2.7	2.4
Significant Difference ($P=0.05$)	18.3	11.1	10.1	8.8	7.7	6.8
AT BOTH STATIONS						
Standard Error %	(8)	(16)	(24)	(32)	(40)	(48)
Expected ($V=11.7$)	4.1	2.9	2.4	2.1	1.8	1.7
Obtained	4.2	2.7	2.5	2.2	1.9	1.7
Significant Difference ($P=0.05$)	12.9	7.8	7.2	6.2	5.4	4.8

In Table IV the results from both stations are analysed together, and the resulting standard errors obtained compared with those expected. As will be seen, the agreement with expectation is closer here, as now even where there is only one square at each station the coefficient of variability is calculated from quite a considerable number of plots.

If the varietal means at each station are compared, the accuracy is intermediate between that obtained where the two single stations are analysed separately; but if the varietal means for both stations together are compared, a considerable increase in accuracy is obtained. Thus, with one square at each of two stations, a difference of about 13% in average yield would be significant; with two squares, about 8%, with a further reduction to 5% where all six squares are used.

In Table V the data from all three stations are analysed together from one to four squares. In this case the coefficient of variability on which the expectations are based is rather higher, since plots at both Long Ashton and East Malling were more variable than at Wisley.

TABLE V.

Comparison of precision expected and obtained by increasing numbers of squares when three stations are analysed together.

Number of Squares Number of Replications	1 (4)	2 (8)	3 (12)	4 (16)
AT EACH STATION				
Standard Error %				
Expected ($V=12.6$)	6.3	4.5	3.6	3.2
Obtained	7.8	3.9	3.8	3.2
Significant Difference ($P=0.05$)	23.2	11.1	10.7	8.9
AT ALL THREE STATIONS	(12)	(24)	(36)	(48)
Standard Error %				
Expected ($V=12.6$)	3.6	2.6	2.1	1.8
Obtained	4.5	2.2	2.2	1.8
Significant Difference ($P=0.05$)	13.5	6.4	6.2	5.1

There is a fairly wide deviation between the standard errors expected and obtained for one square, illustrating the comparatively low reliability of the coefficient of variability where numbers are small. For the rest, the significant differences between means at individual stations are about the same as in Table IV, but there is a further increase in precision when the means of the four varieties at all three stations together are compared. Two squares at three stations will prove a difference of about 7% and four squares will give about the same precision as six squares at two stations.

To sum up, it seems likely that from three to four four by four Latin squares would be ample for a single experiment in one place to show differences of the order of 10%. If the experiment were repeated at two or three stations, two squares, i.e. eight replicated four-bush plots, would be enough to prove definitely the existence of differences in the mean performance at all stations of 8%; while if the varieties do not respond alike to the varying soils and climates in different places, differences at individual stations of 13% would be significant.

ACKNOWLEDGMENT.

This report has been written, following the analysis of the data by the writers, at the request of the Royal Horticultural Society. The data were collected and recorded by the staffs of the three stations concerned, and the writers gratefully acknowledge their indebtedness to them.

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SUMMARY.

The yield data of four varieties of black currant over a five-year period secured from an extensive experiment carried out simultaneously at three stations are examined.

It is shown that the experimental design used, namely six four by four Latin squares with a four-bush plot as the experimental unit, gave exceptionally accurate results. Large differences were shown not only between the gross varietal yields at all stations, but also in the response at individual stations.

These data were used to determine the best number of replicated plots for use in such trials. It is concluded that whereas from three to four four by four Latin squares would be necessary for reasonable precision at a single station, two similar squares would be ample if the experiment were repeated at two or three stations.

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LABORATORY METHODS FOR THE BIOLOGICAL TESTING OF INSECTICIDES

I. METHODS OF TESTING OVICIDES

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W.S.

INTRODUCTION.

THE number of materials requiring consideration as ingredients of horticultural sprays has vastly increased in recent years. From such a host of potential insecticides the few worthy of close investigation can be sorted out only by rapid and reliable laboratory methods of testing. Accurate biological tests are also called for in any attempt to correlate chemical constitution with insecticidal action and thereby rationalize the search for new insecticides and new combinations of spray materials.

Laboratory tests of a fairly rough type have long been employed at East Malling to gain preliminary information concerning the behaviour of insecticides intended for field trial, but the need for more refined methods led, in 1933, to the start of a special study of the whole problem.* The aim throughout has been to devise simple methods that will give reproducible results in tests of ovicides, contact insecticides and stomach poisons. Two main considerations have influenced the development of these methods. These are, the necessity for standardizing both the biological material and every stage in procedure, and the desirability of using as test insects only such species as are representative of the several types commonly occurring as pests of fruit trees and bushes.

Experience gained in this investigation has shown that the biological testing of insecticides is best carried out in four stages. These are:

- A. Preliminary, successive "sorting tests" carried out in the laboratory.
- B. Investigation of the phytocidal properties of the more promising materials selected in stage A.
- C. Small field trials, followed by larger ones, of the more promising of the substances that stage B has shown can safely be applied to fruit trees and bushes.
- D. Field trials of selected substances as components of the routine spray programme.

The scope of the present paper is confined to stages A and B in so far as they concern ovicides.

* The following have at various times taken part in these investigations: Messrs. G. L. Hey, F. J. D. Thomas and G. A. Brett. To them, and particularly to Mr. Thomas, credit is due for devising many details of the methods that have been developed.

THE CHOICE OF TEST INSECTS.

Species selected for regular use in laboratory trials should satisfy as many as possible of the following requirements:

1. *Ease of rearing.* They should pair readily in captivity and lay eggs in conveniently arranged groups and in accessible positions. They should show no tendency to cannibalism and they should be hardy and thus able to withstand frequent handling.

2. *Ease of handling.* Some insects can be picked up with a brush more readily than others and can thus be more rapidly transferred to clean cages and fresh food. External feeders are much easier to handle than insects that feed in the interior of stems or that spin leaves together and feed between them. Again, some crawl rapidly and are difficult to retain in the desired position for spraying. For tests of ovicides it is essential to use insects that will lay eggs on muslin, twigs or other material that can readily be treated with an insecticide, and in such a manner that they can be counted accurately and easily.

3. *Ability to make use of a wide range of host plants.* Insects that feed equally well on several different host plants are to be preferred, since it may often prove difficult or impossible to obtain adequate fresh supplies of a particular plant whenever required.

4. *Availability at convenient periods of the year.* To avoid clashing with the seasonal operations of field spraying and recording, the laboratory tests have to be carried out as far as possible at other periods of the year. It is desirable, therefore, that large stocks of test insects should be obtainable at those periods when they can most conveniently be dealt with. This object can sometimes be attained by suitable adjustment of the temperature at which the insects are maintained.

5. *Suitability for use as dual purpose insects.* A species that can be used not only for ovicide but also for contact or stomach poison tests is economical of labour and equipment, since only one stock need be maintained for the several purposes.

6. *Economic importance.* The insects used should preferably be species that commonly occur as pests or are typical of common pests in their resistance to insecticides.

Whenever possible specially reared insects should be used, particularly for tests of contact insecticides and stomach poisons, since they are free from parasites and are more uniform in development than field-collected specimens. For tests of ovicides, field-collected eggs of certain species, e.g. *Aphis pomi* and *Oligonychus ulmi*, can well be employed, provided healthy parasite-free stocks can be obtained.

At East Malling, the ovicide tests have been carried out chiefly on eggs of the Vapourer Moth (*Orgyia antiqua* L.), the Winter Moth (*Operophtera brumata* L.), Green Apple Aphis (*Aphis pomi* de G.) and Red Spider (*Oligonychus ulmi* Koch).

VAPOURER MOTH.

The Vapourer Moth is a very useful test insect, since it is easily reared and handled and can be used as a dual purpose insect. The eggs, which are laid in convenient batches of two to three hundred, can be used for ovicide tests during the winter, whilst the larvae can be used for tests of contact insecticides and stomach poisons in the spring and summer. Under normal conditions the eggs are laid in August or September and hatch in the following May, but the periods during which eggs and larvae are available can be considerably lengthened. Thus, hatching can be delayed till August if the eggs are kept at a low temperature (5° C.) until a fortnight before the desired hatching time, whilst larvae can easily be obtained in December by incubating the eggs at 21° C.

Raising Stocks of Eggs.

An adequate stock of eggs can be raised within a year, from a nucleus of half a dozen wild batches which can be collected without much difficulty in almost any orchard. The larvae can be reared on foliage of Myrobolan, plum, apple, raspberry, privet and many other plants.

At East Malling the newly hatched larvae are kept in the laboratory in cages made up of hurricane-lamp chimneys and pots of sand (Pl. 2, Fig. 1). Shoots of Myrobolan are inserted in the sand, which has previously been wetted, and then enclosed by the lamp-glasses. About two hundred newly-hatched larvae are placed in each cage, the top of which is then covered with a piece of muslin secured by an elastic band. For the very young larvae a much finer material such as organdie is advisable, but older larvae can easily be retained with a cheap grade of butter muslin. The cages are then arranged on shelves, the pots standing in saucers to which water can be added as required. The amount of water that must be added can be determined after a little experience—it must be sufficient to keep the foliage from wilting but not enough to cause undue condensation on the glass.

To avoid excessive mortality amongst the larvae, the food is renewed frequently. It can be kept in a fresh state for about a week, but is best changed after about four days. Whenever the food is changed the larvae are transferred to a fresh cage. This enables a count to be made and facilitates the removal of the larval excrement which would otherwise encourage the growth of moulds.

When the larvae have reached their second or third instar they are transferred to an outdoor insectary. There they are kept in a large cage, made of wood and wire gauze, and fed on *Myrobolan* shoots, the cut ends of which are thrust into wet sand on the floor of the cage.

When fully fed, the larvae will, if left undisturbed, crawl to the roof of the cage and spin up in the topmost corners, where large masses of cocoons will soon accumulate. To avoid this, the fully-fed larvae are removed to a "finishing cage". There they are placed on a few shoots standing in sand and surrounded by four wooden boards. To the inner surfaces of the boards, paper folded into the shape of shelves is attached (Pl. 1, Fig. 2). Most of the larvae spin their cocoons on the paper shelves, and these can be removed before they become too full of cocoons and hung up in some convenient place until the moths emerge. The few larvae that reach the roof are allowed to pupate on strips of paper pinned to the framework of the cage.

After emergence the female moths remain on the outside of their cocoons, since they cannot fly, to await fertilization. The egg-batches are then laid on the cocoons or, occasionally, on the paper to which these are attached. Periodically, the batches are collected, graded, and stored in suitable containers. As there is no means of detecting infertile batches by the appearance of the eggs, it is advisable always to ascertain whether plenty of males are present, and to discard any batches that may have been laid in the absence of them.

Conducting the Tests.

The method adopted is based on one already used in 1926 and 1927 (2, 4), in that the principle is followed of setting aside a portion of each egg-batch to serve as a control and as a check on the fertility of the eggs. The present procedure differs greatly, however, from that formerly employed; it is standardized, and enables quick results to be obtained, whilst the improved methods of rearing and handling the insects have resulted in a saving of labour and in greater fertility.

Only the best of the egg-batches are used for ovicide tests; small batches and those containing collapsed or otherwise unsuitable eggs are discarded. Every batch is divided into four approximately equal portions, each of which can be dealt with separately and so enable as many as three insecticides to be tested on the same egg-batch. At least six replications, i.e. six batches comprising some fifteen hundred eggs, are used in a test.

Each quarter-batch is sprayed with the appropriate concentration of the material to be tested and placed in a small glass dish to drain. When dry it is placed in a small glass tube ($\frac{1}{2}$ inch diameter), the open end of which is then closed with a piece of organdie, held in place by a drilled cork or a piece of rubber tubing. A label bearing the egg-batch number (which enables the

history of the batch to be readily ascertained) and a letter representing the spray treatment is placed in the tube.

All the tubes of one test are packed in a shallow cardboard box, bearing the serial number of the test, and put into an incubator maintained at 21° C.

The compact arrangement of the tubes and the simple system of labelling enable the most to be made of limited incubator accommodation and facilitate handling and recording.

Hatching begins after 10-14 days—although eggs put in as early as November may take three weeks—and continues, usually, for 6-7 weeks. During the hatching period larvae are removed from the tubes every few days.

The eggs are counted with the aid of a binocular microscope at any time after hatching is finished. In addition to counts of hatched and unhatched eggs, it has been found convenient to record also the number of partly hatched eggs (i.e. those in which the larvae pierce the chorion but die before hatching properly), and of eggs collapsed or discoloured as a result of spray treatment.

A method of calculating the result of a test from the numbers of larvae that hatch out is often adopted to obtain an interim result on which to base further experimental work without having to wait for the final egg counts. The per cent. kill is reckoned as

$$100 - \frac{100 \times (\text{number of larvae hatched in sprayed series})}{\text{number of larvae hatched in control series}}$$

This method is based on the assumption that the number of eggs used is approximately the same in each treatment, and gives results that agree very closely with those ultimately obtained from egg counts (Table I).

Laboratory ovicide trials with Vapourer Moth eggs stored under outdoor conditions can be undertaken during the months December to April inclusive, and, since an interim result can, as in the example quoted, be obtained within a month, as many as four consecutive interdependent series of tests can be carried out in a single season.

For treating the eggs with an insecticide a Tattersfield apparatus (5) is used. Such a machine is not essential, since satisfactory results can be obtained if the eggs are merely dipped in the insecticide. It was originally obtained for testing contact insecticides, but has since proved a quick and convenient means of applying insecticides to Vapourer Moth egg-batches, which can by its aid be dealt with at the rate of more than two a minute.

The apparatus is worked in a similar manner to that originally described by Tattersfield and Morris, but the dish of material to be treated is placed in a rectangular glass-sided box instead of in a cylindrical glass jar. An aperture in one side enables the spraying to be carried out rapidly and obviates repeated removal of the lid. The atomizer has been adequately described by Tattersfield (6). Two operators are needed. One prepares and applies the insecticides ;

TABLE I.
Showing the agreement between interim and final results in a laboratory test
 (No. 0.70/35) on *Vapourer Moth* eggs.

Treatment.*	First interim result based on larval counts up to February 14th, 1936, i.e. two weeks after hatching began.		Second interim result based on larval counts up to April 6th, 1936, i.e. at end of hatching period.		Final result based on egg counts made on March 2nd, 1937.		
	No. of larvae hatched.	% Reduction of hatch.	No. of larvae hatched.†	% Reduction of hatch.	Total No. of eggs.	No. of eggs hatched.†	% viable eggs killed.
A. 4% emulsion of a tar oil B.P. 250-300° C.	30	85.6	67	81.9	409	73	80.9
B. 4% " " 300-350° C.	7	96.7	27	92.7	385	27	92.5
C. 4% " " > 350° C.	65	68.9	150	59.6	377	151	56.9
D. Untreated Controls " " "	209	—	371	—	401	373	—

* In this, as in other examples of laboratory tests given below, details of the composition of the insecticides used are omitted, since they are scarcely relevant to a discussion on biological methods, and will be fully dealt with elsewhere.

† The slight discrepancy between the figures in these two columns is accounted for by a few larvae hatching after April 6th.

the other prepares the eggs, puts them into the apparatus and removes them after they are sprayed. 2 c.c. of spray material is used to each dish of eggs, since, as Table II shows, this amount gives results comparable with those obtained when the eggs are dipped.

TABLE II.

Showing the effect of differences in the method of applying an insecticide to Vapourer Moth eggs.

Treatment.						% * hatch.	S.E.
A.	Eggs sprayed with 1 c.c. of a 4% tar oil emulsion	..	..	..	..	14.1	4.7
B.	" " " 2 c.c. " " " "	..	..	..	..	0.2	0.2
C.	" " dipped in a 4% tar oil emulsion	..	..	..	..	1.6	1.3
D.	" Untreated Controls	..	..	..	..	82.5	7.1

* In these Tables, brackets are used to indicate which differences are insignificant.

WINTER MOTH.

The Winter Moth possesses the obvious merit of being a common fruit pest. Unfortunately the larvae are somewhat difficult to rear and to handle in large numbers. Their cannibalist tendency often causes serious losses, and their habit of feeding enclosed in coiled-up or spun-together leaves makes re-feeding long and tedious.

Raising Stocks of Eggs.

It has been found best to raise stocks of eggs from moths collected on fruit trees in neglected plantations. Where such plantations are not available, it would be advisable to encourage the pest to develop on a small block of fruit trees by infesting them with larvae.

Being wingless, the female moth has to crawl up the trunk of the tree, from the soil wherein she pupated, to lay her eggs on the twigs. The males fly about at night and it is generally thought best to search for the ascending females after dusk. In a neglected plot of apple trees near the Research Station, however, it has never proved difficult to find sufficient moths during the daytime. Over 900 females were collected during the winter of 1936-37, and Fig. 1 shows that these were found during the period November 16th to January 8th, but chiefly during December. A less intensive search, carried out in the previous winter, yielded 130 moths, most of which were found between December 10th and 20th, a few being collected late in December and early in January, but none in November or after January 21st.

Attempts were also made to rear moths from larvae collected on fruit trees during the summer. Since many of the larvae were parasitized and others were

lost as a result of cannibalism, the pupae that developed were disappointingly few. About 150 pupae, reared in 1936, were kept in a cool laboratory, and moths emerged from 65% of them (72% when the pupae were stored in peat moss, and only 50% when they were stored in sand). 70% of the moths were females but, as these often began to lay before pairing could be effected, many of their eggs proved infertile. Emergence was spread over so long a period

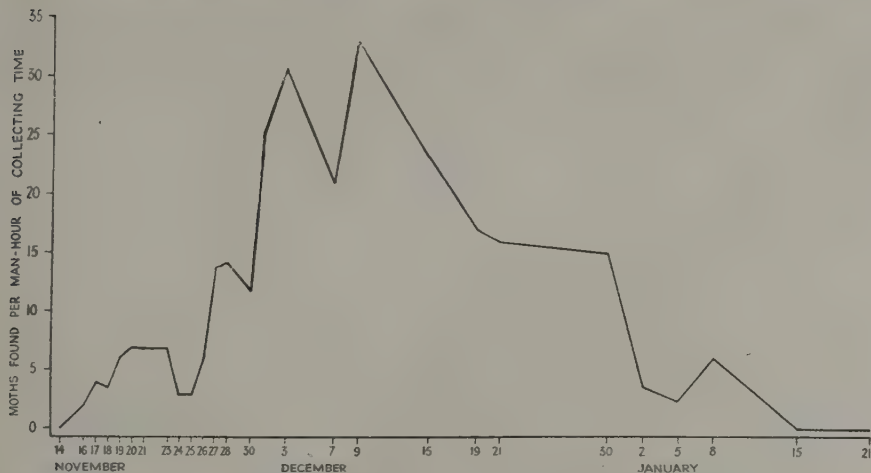


FIG. 1. *O. brumata* females collected in a neglected orchard in the winter of 1936-37.

(Fig. 2) that sufficient eggs for satisfactory ovicide tests could not be obtained at any one time.

Eggs, laid in sufficient numbers and on suitable material for ovicide tests, can be obtained by confining 15-20 female moths on apple shoots in a cage such as that illustrated (Pl. 2, Fig. 1). The eggs are laid on the shoots, mostly at the base of the buds, and also on the muslin cover of the lamp-glass. The

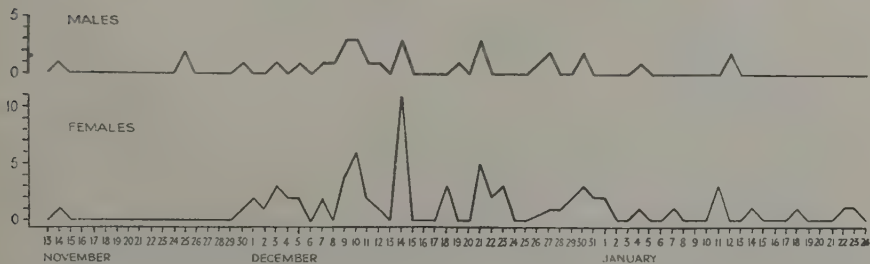


FIG. 2. Emergence of moths from laboratory-reared *O. brumata* pupae, 1936-37.

flower-pot portion of the cage stands in a saucer of water ; this keeps the sand moist and prevents the shoots shrivelling. Very little space is required, and as many as eight of these cages can, if necessary, be placed on a yard of ordinary nine-inch shelving.

The shoots should be clean and free from deep cracks or cankers and well furnished with very short spurs. Satisfactory shoots have been obtained from trees of Lane's Prince Albert that were left unpruned for two seasons with the result that vigorous shoots grew out and later on developed plentiful fruit buds. Old shoots, with dead spurs or loose bark and crevices, should be avoided ; eggs are freely laid on them but are not easily found and counted. On the other hand, moths cannot be induced to lay sufficient eggs on the current year's growths, which are smooth and bear only wood buds, even if cuts or scratches are first made in the bark. Several attempts have been made to get them to lay on butchers' wooden skewers and other artificial substitutes for apple shoots, but without success.

Eggs can be obtained by confining moths in Petri dishes with discs of muslin on the bottoms, but, as so many of the eggs are laid on the glass and therefore of little use, such a method is wasteful. Other receptacles have also been tried. Large paper cylinders were useless, as eggs were seldom laid except in crevices formed by the overlapping edges of the paper. When suspended in muslin bags the moths laid eggs readily, if somewhat unevenly, but soon perished.

A somewhat humid atmosphere seems to be essential to the moths and this is secured by confining them with twigs in lamp jars standing on moist sand. Under these conditions wild females can be expected to live seven days and to produce 40-50 eggs apiece, of which less than 1% are infertile. Bred females are usually more prolific but, even if males are provided, up to 25% of their eggs may be infertile.

Conducting the Tests.

Method 1. Shoots bearing sufficient eggs are selected and dipped, one at a time, in a glass cylinder containing the spray material to be tested. They are then allowed to dry in the laboratory with their basal ends thrust into moist sand. When dry they are placed in jars containing sand and water, suitably labelled (Pl. 2, Fig. 2), and placed in the insect-proof chambers of an insectary. There they are lightly sprayed with water from time to time. Five shoots are normally used for each treatment and can be relied on to bear 50-100 eggs each.

When hatching is finished, the shoots are brought into the laboratory and examined under a binocular microscope. Hatched and unhatched eggs can readily be recognized, but, since many of them are thrust into crevices between

bud and stem or under loose bud scales, careful search is necessary to avoid overlooking some of the eggs. This search can be facilitated by first cutting the shoots up into one-inch lengths and then examining each piece separately. In counting, the total is best reckoned mentally and the number of either hatched or unhatched eggs recorded with a hand tally counter.

The hatched eggs are empty, colourless shells with a hole in one end; the unhatched are either reddish brown or black in colour and may be partly collapsed. If counting is long delayed the contents of some of the unhatched eggs may shrink and become inconspicuous. Care is then needed to distinguish between hatched eggs and such eggs as these, since both have colourless shells.

The result of part of a typical ovicide test is shown in Table III.

TABLE III.

Example of laboratory test (No. 0.14/36) on Winter Moth eggs.

Treatment.			Total No. of eggs.	% hatched.	S.E.
A.	Petroleum oil,	4% emulsion ..	461	0.3	0.3
G.	Tar oil	4% ,, ..	352	8.6	4.1
B.	Petroleum oil,	2% ,, ..	333	20.4	5.0
H.	Tar oil	2% ,, ..	516	23.2	1.9
J.	Soap Controls		513	84.5	3.6
K.	Untreated Controls		367	92.8	1.4

Method 2. Whenever the lamp-glass method of obtaining eggs is employed some eggs are laid on the muslin covers.* Pieces of this muslin, bearing sufficient eggs, are dipped in the insecticide, hung up to dry and placed in 3" x 1" glass tubes, the corks of which are drilled and covered with muslin. These are then placed in an incubator or stored in the laboratory. As the larvae hatch out they are removed and counted, thereby obviating the necessity of counting the hatched eggs later on. The eggs remaining unhatched can best be counted by placing the muslin between two sheets of glass, one of which is marked in centimetre squares in the manner of the Vyvyan Integrator (7). The glass plates hold the muslin flat, and the centimetre divisions facilitate counting.

Eggs laid on apple shoots can also be dealt with in much the same way, provided the shoots are cut into pieces small enough to be placed in glass tubes. Shoots are not entirely satisfactory, however, since they are apt to become mouldy in the incubator. A further objection is that the larvae must be removed and counted almost daily during the hatching period, otherwise

* If eggs laid on shoots only are required, this wastage can be avoided by using cellophane in place of muslin as a means of closing the open ends of the lamp-glasses.

they soon die and shrivel, and so may be confused with small particles of dirt that flake off the twigs.

The muslin method is useful, particularly when quick results are desired, since the device, adopted in the Vapourer Moth ovicide tests, of calculating a result from the numbers of larvae that hatch, can be used. On the other hand, it may not prove as reliable as Method 1, since the muslin is liable to absorb some of the insecticide and so retain it in close contact with the eggs.

Results obtained with the two methods are compared in Table IV. Method 2 gave a lower hatch of treated but a slightly greater hatch of untreated eggs, both differences being significant. Further information on the relative merits of the two methods is being sought.

TABLE IV.

Comparison of two methods of conducting insecticide tests on Winter Moth eggs.

Treatment.	Mean % hatch.		S.E. of difference.	Sig. Diff.
	Method 1.	Method 2.		
Petroleum oil, 2% emulsion ..	20.4	1.5	5.04	11.6
Untreated Control	92.8	98.8	1.66	3.8

GREEN APPLE APHIS.

The Green Apple Aphis feeds and lays its eggs clustered closely together on the current season's growths. It is a convenient species for ovicide tests and, for them, it may safely be regarded as similar in behaviour to other aphides that overwinter as eggs on fruit trees and bushes.

Collection of Egg Material.

Supplies of eggs, which are laid in October, November and December, can most conveniently be obtained from apple rootstock stool-beds. If such beds are maintained solely for this purpose, the stocks should not be cut till January, since aphides persist and eggs are laid up to the end of December. At East Malling, the rootstocks are normally lifted in November and December, and prepared for sale. Aphis infested shoots required for laboratory tests have, therefore, to be cut out in November. Since these are not required for use till January they are heeled-in in an enclosure protected from sparrows and other birds by walls and roof of $\frac{1}{2}$ " mesh galvanized wire netting. Well infested stocks are chosen, the soil is removed from the base and the stems are cut below soil level leaving some roots attached. Great care is needed in lifting

and transporting the stocks, but, if they are grasped only by the roots, they can be handled without damage either to the aphides or the eggs.

Good colonies of eggs have been found in stools of Malling Types II, XVI and XII, each of which produces long straight growths, convenient to handle and well suited to the procedure followed in carrying out the tests. Shoots of Types I and IX have usually had to be rejected on account of poor infestation or shortness of growth.

The long vigorous shoots that are produced by heavily pruned trees are liable to attack by *A. pomi*, and will often provide suitable egg material. Stool-beds, however, are more reliable as a source of supply.

Conduct of the Tests.

The ingenious method described by Kearns and Martin (3) is now used for all toxicity trials on *A. pomi* eggs. The procedure has been somewhat modified to suit East Malling facilities and to bring it into line with that followed with other eggs, such as those of Winter Moth and Red Spider. Formerly, the results of tests carried out on *A. pomi* eggs could be assessed only by counting the hatched and unhatched eggs that could be seen in limited fields of view under a binocular microscope, since they are so closely crowded together as to make it impossible to count all the eggs on a twig. Now, however, all the eggs can be removed from the twigs and counted with reasonable accuracy.

When all preparations have been made for carrying out a test, the heeled-in stocks are cut (as near the base as possible) with secateurs and brought into the laboratory. They are handled by their basal ends which usually bear very few eggs and are in any case always discarded. In the laboratory they are carefully examined and the best of them cut into ten-inch lengths. Shoots bearing collapsed eggs and those portions that are cankered or killed by aphid attack are discarded.

The pieces of shoot are dipped, one at a time, in a 250 c.c. graduated cylinder filled to the brim with 300 c.c. of spray fluid. Thorough wetting is ensured by dipping, lifting and redipping each shoot and then repeating the same movements with the shoot reversed. The total immersion time is about ten seconds. The shoots, five of which are used for each treatment, need be handled only by the ends, these being discarded when the final sampling is carried out.

After being dipped, the shoots stand in the laboratory to dry, with their basal ends thrust into moist sand in a wooden box. They are then transferred to jars of wet sand (Pl. 2, Fig. 2). The five shoots of one treatment easily fit into one jar, which has the appropriate test number and treatment letter painted on it. A large number of such jars can be stored in a small insect-proof compartment of the insectary. Water is added to the jars as often as is necessary and occasionally the shoots themselves are lightly sprayed with water.

All tests, with the exception of special time of treatment trials, are carried out in January and February, and the above method of dipping and handling has proved simple, rapid and convenient.

The eggs begin to hatch at the end of March. Just before this happens small samples are cut from the twigs and placed in $4\frac{1}{2}'' \times 2''$ glass tubes (Pl. 1, Fig. 1). These stand on shelves in a cool laboratory until all the aphides that hatch out are dead.

TABLE V.

Example of laboratory test (No. 0.8/36) on A. pomi eggs.

Treatment.	Twig No.	No. of eggs used.	% hatch.	Mean % hatch.	S.E.
C. Nicotine 0.1% + Soap ..	1	322	0	0	0
	2	112	0		
	3	287	0		
	4	206	0		
	5	137	0		
D. Nicotine 0.07% + Soap ..	1	470	20.6	20.4	0.6
	2	321	21.5		
	3	104	18.3		
	4	97	21.6		
	5	192	19.8		
E. Nicotine 0.04% + Soap ..	1	203	38.4	32.8	1.6
	2	285	30.2		
	3	172	29.1		
	4	86	33.7		
B. Soap Controls	1	344	97.4	88.4	3.8
	2	383	95.0		
	3	186	75.3		
	4	410	88.5		
	5	120	85.8		
F. Untreated Controls	1	235	94.5	94.2	0.5
	2	232	94.0		
	3	820	95.7		
	4	291	94.2		
	5	68	92.6		

It has been found that mould readily develops on shoots kept in tightly corked tubes. If, however, a large hole is drilled through each cork the shoots dry out without harm to the eggs. The aphides are prevented from escaping by a piece of fine muslin or organdie held in place by the cork. Again, in cutting the samples, it is better to cut off portions that can be seen to bear about 300 eggs, which may be anything from one to four inches long, rather than to cut standard lengths of, say, two inches entirely at random. Excessive variation in the numbers of eggs used is thereby avoided.

To remove the eggs from the shoots, these are warmed in a test tube containing 10% caustic potash for ten minutes, the fluid poured off and filtered ; further warm caustic potash is added and poured off in a similar manner until eggs can no longer be removed. The shoot is finally shaken vigorously in a test tube of water to determine whether any eggs remain. The filtrate can be used several times. The eggs are washed off the filter papers with water and counted in a Petri dish standing on squared paper. The dead aphides are shaken out of the tubes and counted to determine the number of eggs that hatched.

The result of part of a typical test is shown in Table V.

RED SPIDER.

Although in many ways unsatisfactory for laboratory use, Red Spider has some value as being an example of that small group of pests susceptible to petroleum oil but unaffected by tar oil sprays.

Source of Eggs.

The winter eggs are laid on twigs and branches of apple, plum and other fruit trees. They are rarely laid on the current year's wood, so that the older shoots and spurs form the only material that can well be used in laboratory tests. There is little difficulty in obtaining adequate supplies of eggs since, in most districts, Red Spider rapidly increases on trees that are not sprayed regularly with lime-sulphur, and particularly on trees that are annually sprayed with a tar oil winter wash.

Shoots bearing eggs can be cut and kept in the insectary or in the laboratory with the cut ends immersed in wet sand or water. Provided the shoots are moistened from time to time by spraying them with water, the eggs hatch satisfactorily.

Conduct of the Tests.

Method 1. Shoots bearing eggs are dipped into the spray fluid to be tested, drained and stood in jars of sand and water on a bench to await hatching. When hatching is complete, counts are made, with the aid of a binocular microscope and a mechanical hand counter, of hatched and unhatched eggs, on short lengths of shoot chosen at random. In almost any sample of Red Spider eggs, some shells of previous seasons' eggs can be found, still attached to the bark. As it is usually impossible to differentiate between these old shells and those of newly hatched eggs, the counts are not entirely satisfactory as an index of the degree of control. The number of old shells present has relatively more effect on results obtained with sprays that give a high kill than on those obtained with sprays of indifferent efficiency.

One method of overcoming this difficulty is to estimate the proportion of old shells in the egg population by adequate counts of random samples, made before the beginning of hatching. The actual percentage hatch is then reckoned as $\frac{100(H-S)}{100-S}$, where H is the apparent per cent. hatch and S is the estimated percentage of old shells present. Thus, supposing egg counts indicate apparent hatches of 24% and 92% on sprayed and unsprayed shoots, and that the proportion of old shells has previously been estimated at 20%, then the actual hatch in the two series would be $\frac{100(24-20)}{100-20}\%$ and $\frac{100(92-20)}{100-20}\%$, i.e. 5% and 90%, respectively. Frequently, however, the proportion of old shells varies so greatly from shoot to shoot that it can be estimated only within very wide limits, when the correction cannot well be applied.

Data obtained by this method in a small scale test on Red Spider eggs are summarized in Table VI.

TABLE VI.

Example of laboratory test (No. 0.97/35) on Red Spider eggs. Method 1.

Treatment.		No. of eggs used.	% hatch.	S.E.	Calculated " actual " hatch, %
A.	Petroleum oil, 6% emulsion ..	580	30.1	2.8	0
B.	" " 4% " ..	400	33.2	3.0	4
C.	" " 2% " ..	400	30.0	4.9	0
D.	Tar oil " 6% " ..	400	68.0	7.4	54
K.	Untreated Controls ..	330	89.5	2.5	85

Although counts of random samples of eggs indicated a moderately high apparent hatch in A, B and C, no mites were observed to hatch in A and very few indeed in B or C. It is evident, therefore, that some 30% of the eggs were old shells. Using this figure to calculate the actual hatch, the result shown in column 5 is obtained. This agrees closely with notes made at hatching time on the degree of control given by the various treatments, but the uncorrected data (columns 3 and 4) are, in this example, adequate for tests of significance.

Method 2. As the above method is in many ways unsatisfactory, a modification of the procedure has been devised but has not yet been thoroughly tried out. From the shoots used in a test, random samples $\frac{1}{2}$ " to 1" long are cut off and placed in glass tubes fitted with drilled corks. The mites hatch out but are prevented from leaving the tubes by a piece of closely woven material such as calico, which, however, allows sufficient ventilation to prevent mould

growing on the cut shoots. After hatching, the mites can be counted, with some difficulty, and the unhatched eggs can be removed from the shoots with caustic potash and counted in the manner adopted for *A. pomi* eggs. The old shells are not counted and are thus of no consequence.

An experiment designed to compare the above two methods of testing ovicides on *O. ulmi* was undertaken in 1937. This failed, since, for some unknown reason, hardly any of the eggs hatched, even on the trees from which the shoots were taken.

Carroll (1) has described a method of conducting laboratory trials on Red Spider eggs. This, however, necessitates much tedious counting of mites and eggs and the resulting data are not altogether convenient for statistical treatment.

TESTING FOR SPRAY DAMAGE.

Before new or little known insecticides are used in field trials it is important that something should be known of their phytocidal properties.

The more promising of the materials undergoing tests in the laboratory are, therefore, tried out at suitable concentrations on established fruit trees and bushes. It is convenient to use a single large branch of a tree as a unit. Several sprays can then be tested on the same tree, thereby avoiding the necessity of using (and possibly destroying) a large block of valuable trees.

Selected branches are sprayed by means of a knapsack machine or, if the amount of spray material available is small, with a small double-action hand-pump sprayer. Care is taken to wet all parts of the branch thoroughly, and the spray is prevented from reaching the rest of the tree by large pieces of sack hung over the neighbouring branches.

Ovicides are tested at both dormant and semi-dormant stages in the development of the tree, and, at the time of spraying, notes are made on such incidental matters as ease of mixing, wetting power and rate of drying of the various sprays. After spraying, periodical observations are made on the effect on the development of the buds. These tests are carried out chiefly on Lord Derby, Lane's Prince Albert and Cox's Orange Pippin apples, and on Victoria, Pond's Seedling, Monarch and Czar plums.

Materials that cause injury at concentrations high enough to kill insect eggs are usually not further considered. Those that cause no injury are used, first in small-scale then in larger, field trials where, in addition to their effect on particular pests, any tendency to cause injury to the trees is noted. No new material can be regarded as safe for use in commercial orchards until it has undergone field trials for several seasons, since differences of season, soil, variety and rootstock often have a great, though little understood, influence on the propensity of fruit trees to suffer spray injury.

The single branch system can seldom be used on bushes and never on such plants as strawberries. For phytocide tests on these, individual plants or sets of plants must be used as units, and the effect of any normal variation in susceptibility provided for by adequate replication of the treatments.

SUMMARY.

Standardized methods of conducting biological tests with suitable insect species as indicators are indispensable for the rapid sorting of new and potential insecticides and for the correlation of toxicity and chemical constitution. Careful attention to details of rearing and handling is necessary to eliminate all but inherent sources of variation in the insects used.

For testing ovicides the eggs of *Orgyia antiqua* L., *Operophtera brumata* L., *Aphis pomi* de G. and, to a lesser extent, *Oligonychus ulmi* Koch, are used.

O. antiqua is bred in large numbers and is useful for tests of contact and stomach poisons in addition to ovicides. After insecticidal treatment the eggs are incubated at 21° C. to accelerate hatching and to get early results.

Females of *O. brumata* are collected in orchards and induced to lay eggs on twigs in special cages. The twigs are then dipped in the insecticide and the result assessed from subsequent counts of hatched and unhatched eggs. Eggs laid on muslin can also be utilized if the testing procedure is suitably modified.

Shoots bearing eggs of *A. pomi* are collected from apple rootstock stool-beds and treated by an adaption of the Kearns and Martin method.

O. ulmi eggs can be used but are not altogether satisfactory.

Spray materials that show sufficient promise in laboratory tests are applied to branches of fruit trees to ascertain whether they are safe to use. If satisfactory, they then undergo successive stages of field trial.

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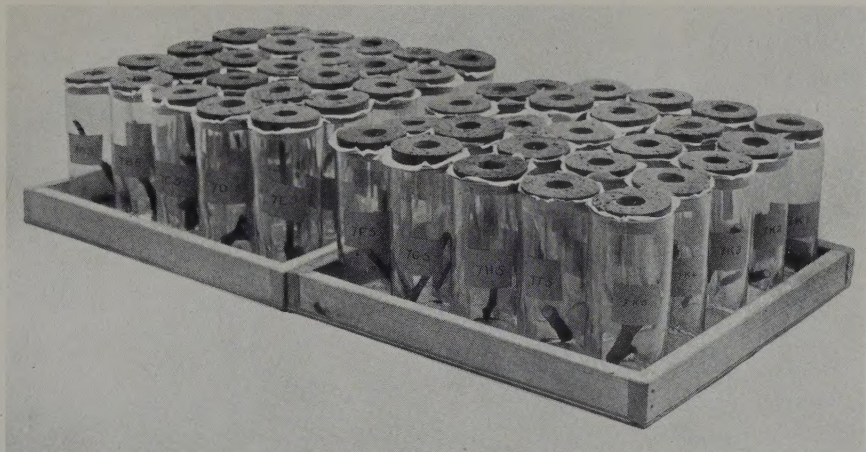


FIG. 1. Shoot samples in an *A. pomi* ovicide test. Aphides can be seen in some of the tubes.

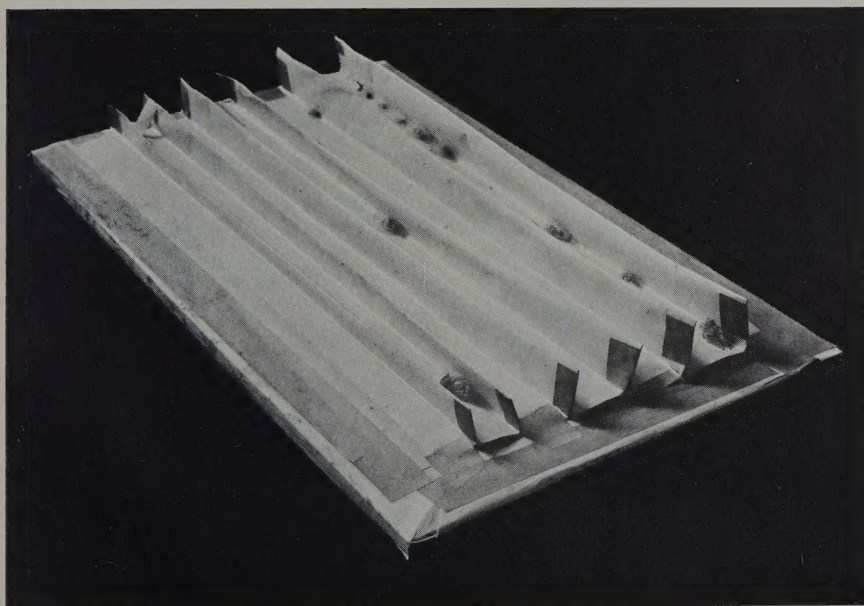


FIG. 2. Device for obtaining Vapourer Moth cocoons in a convenient form.

PLATE II

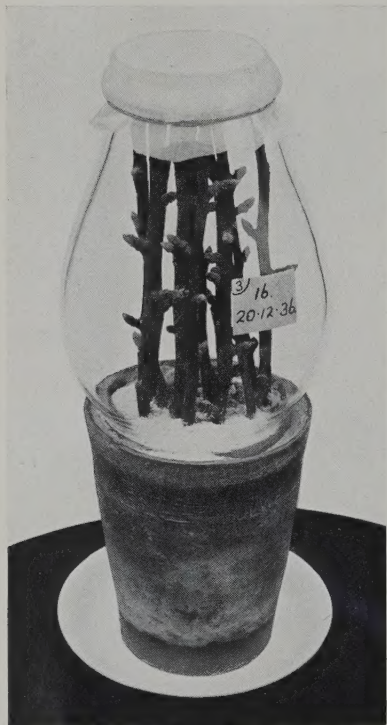


FIG. 1. Cage used for oviposition by Winter Moth (*Operophtera brumata* L.) females. Similar cages are used to confine small Vapourer Moth larvae on Myrobolan foliage.



FIG. 2. Twigs bearing eggs of *Aphis pomi* de G. that have been dipped in an insecticide, ready for removal to the insectary. A similar arrangement is adopted for twigs bearing eggs of Winter Moth and of Red Spider.

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BOOK REVIEWS

THE DESIGN OF EXPERIMENTS. By R. A. FISHER, Sc.D., F.R.S. (Edinburgh and London: Oliver & Boyd. Second Edition. 1937. Price 12s. 6d. net.)

Since Dr. Fisher's first book appeared twelve years ago, a vast amount of interest has grown up concerning the design and interpretation of experiments. During this time a volume of literature on this subject has accumulated, which has greatly outgrown the single chapter devoted to it in that book; so much so that an authoritative book from the originator, devoted exclusively to the application of modern statistical ideas to the conduct and interpretation of experiments, was badly needed. In 1935 this need was met by the publication of the present book, the second edition of which has now appeared.

To say that every research worker would do well to study the opening chapters of this book, whether he be at the start of his career, or in a position with many years of research behind him, is hardly an overstatement. Too often experimental designs betray muddled thinking and a lack of appreciation of the fundamental principles which underlie all experiments, even though the methods of experimentation may be good ones. In horticulture this is especially true, for it has happened all too frequently in the past that a lack of clear thinking in the preliminary stages of a long term field trial has led to years of wasted energy and, in the end, to meaningless results.

This book should go far to place matters on a sounder footing, for in it are set forth clearly and logically the principles of experimental design, showing how once a problem is formulated, an experiment may be planned so as to answer, with any desired precision, definite questions propounded in order to throw light upon it.

Having described, the fundamental ideas behind all experiments the author goes on to develop and explain the various methods of arranging an experiment, associated with his name, and now in common use, ranging from simple randomized blocks and Latin squares to much more complex designs embodying the complete or partial confounding of minor issues in the trial. These latter are built up on the basis of factorial design, the principles of which are explained in Chapter VI.

Here a word of warning should be said, for, while to the inexperienced some of these more complex arrangements are extremely fascinating, they are to be used with the utmost care and circumspection. It is of little use to take a razor to fell a tree; and there are as yet few horticultural crops in which the material available for experiment is sufficiently standardized for these refined tools to be used without discretion.

This is not to say that these methods of experimentation are not exceedingly valuable in their proper sphere; but, for the most part, the horticulturist would do better to adhere to the simpler forms of plot arrangement, such as randomized blocks, until such a time as he is looking for much finer differences between the treatments in his experiment than are usual to-day.

The book concludes with a discussion of tests of significance and precision and the measurement of amount of information in general. No doubt it will become in time the vade-mecum of practical experimenters, perhaps to an even greater extent than its forerunner, which is already to be found on the desks of research workers all over the world. T.N.H.

COMMERCIAL APPLE GROWING. By A. H. HOARE. (Martin Hopkinson Ltd., London, 1937, 237 pp. Price 10s. net.)

This book deals exclusively with the growing, packing and marketing of apples on a commercial scale in this country.

On the hypothesis that of the apples consumed in the raw state in the United Kingdom only one-quarter are at present produced by the home grower, the author considers that there is ample scope for the production of high grade dessert apples here. He urges growers to aim at producing 70 per cent. of combined extra fancy and fancy grades, and recommends a production unit large enough to maintain a minimum output of 50 bushels a day during the marketing season, of not more than four good varieties. The main commercial varieties are briefly described and sound advice is given on planning and planting. Seven years are regarded as the period which may be expected to elapse before large bush trees on stocks of medium vigour can be expected to show any substantial returns. Pruning, manuring and soil management are adequately dealt with in separate chapters. The author has some interesting things to say on certain other factors affecting tree performance, such as June drop, bark ringing, biennial bearing and fruit thinning, but he is careful to point out that these matters are still under investigation.

Full details are given of a modern form of orchard heater, and packing, marketing and storage methods are described accurately and with a marked economy of words. The chapters on the control of pests and diseases contain an excellent description of spraying technique and provide some interesting costing figures. The author maintains that a grower should be in a position to spray efficiently the whole of his acreage in from 3-5 days. The section on finance and accounts and the author's personal note at the end of the book deserve careful study by every prospective fruitgrower. An appendix contains a spray calendar and some facts and figures which should prove useful for reference. This is a valuable textbook about a specialized industry; it is written for business men by a specialist who knows what he is writing about and who has made full use of the findings of science and practice. N.B.B.